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CELLULASE VARIANTS

TECHNICAL FIELD

The present invention relates to cellulase variants derived from a parental cellulase by substitution, insertion and/or deletion, which variant has a catalytic core domain, in which the variant at position 5 holds an alanine residue (A), a serine residue (S), or a threonine residue (T); at position 6 holds a threonine residue (T); at position 7 holds an arginine residue (R); at position 8 holds a phenylalanine residue (F), or a tyrosine residue (Y); at position 9 holds a phenylalanine residue (F), a tryptophan residue (W), or a tyrosine residue (Y); at position 10 holds an aspartic acid residue (D); at position 119 holds a holds a histidine residue (H); and at position 121 holds an aspartic acid residue (D).

BACKGROUND ART

Cellulases or cellulolytic enzymes are enzymes involved in hydrolyses of cellulose. In the hydrolysis of native cellulose, it is known that there are three major types of cellulase enzymes involved, namely cellobiohydrolase (1,4-β-D-glucan cellobiohydrolase, EC 3.2.1.91), endo-β-1,4-glucanase (endo-1,4-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4) and β-glucosidase (EC 3.2.1.21).

Especially the endoglucanases (EC No. 3.2.1.4) constitute an interesting group of hydrolases for the mentioned industrial uses. Endoglucanases catalyses ndo hydrolysis of 1,4-β-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxy methyl cellulose and hydroxy ethyl cellulose), lichenin, β-1,4 bonds in mixed β-1,3 glucans such as cereal β-D-glucans or xyloglucans and other plant material containing cellulosic parts. The authorized name is endo-1,4-β-D-glucan 4-glucano hydrolase, but the abbreviated term endoglucanase is used in the present specification. Reference can be made to T.-M. Enveri, "Microbial Cellulases" in W.M. Fogarty, Microbial Enzymes and Biotechnology, Applied Science Publishers, p. 183-224 (1983); Methods in Enzymology, (1988) Vol. 160, p. 200-391 (edited by Wood, W.A. and Kellogg, S.T.); Béguin, P., "Molecular Biology of C llulose Degradation",

Annu. Rev. Microbiol. (1990), Vol. 44, pp. 219-248; Béguin, P. and Aubert, J-P., "The biological degradation of cellulose", FEMS Microbiology R views 13 (1994) p.25-58; Henrissat, B., "Cellulases and their interaction with cellulose", Cellulose (1994), Vol. 1, pp. 169-196.

Cellulases are synthesized by a large number of microorganisms which include fungi, actinomycetes, myxobacteria and true bacteria but also by plants. Especially endoglucanases of a wide variety of specificities have been identified

A very important industrial use of cellulolytic enzymes is the use for treatment of cellulosic textile or fabric, e.g. as ingredients in detergent compositions or fabric softener compositions, for bio-polishing of new fabric (garment finishing), and for obtaining a "stone-washed" look of cellulose-containing fabric, especially denim, and several methods for such treatment have been suggested, e.g. in GB-A-1 368 599, EP-A-0 307 564 and EP-A-0 435 876, WO 91/17243, WO 91/10732, WO 91/17244, PCT/DK95/000108 and PCT/DK95/00132. Another important industrial use of cellulolytic enzymes is the use for treatment of paper pulp, e.g. for improving the drainage or for deinking of recycled paper.

It is also known that cellulases may or may not have a cellulose binding domain (a CBD). The CBD enhances the binding of the enzyme to a cellulose-containing fiber and increases the efficacy of the catalytic active part of the enzyme

Fungi and bacteria produces a spectrum of cellulolytic enzymes (cellulases) which, on the basis of sequence similarities, can be classified into families. One of these families is known as the cellulase family K, or as the glycosyl hydrolases family 45 [*Henrissat B & Bairoch A*; Biochem. J. 1993 **293** 781-788]. This family includes the following enzymes: Endoglucanase 5 from *Humicola insolens*, endoglucanase 5 from *Trichoderma reesei*, and endoglucanase B from *Pseudomonas fluorescens*.

Cellulases the glycosyl hydrolase family 45 are described in e.g. WO 91/17243, WO 91/17244 and WO 91/10732, and cellulose variants of the glycosyl hydrolase family 45 are described in WO 94/07998.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide nov l variants of cellulolytic enzymes, which variants, when compared to the parental enzyme, show improved performance. More particular the invention provides cellulase variants improved with respect to increased catalytic activity; and/or altered sensitivity to anionic tensides; and/or altered pH optimum.

Accordingly, in its first aspect, the invention provides a cellulase variant derived from a parental cellulase by substitution, insertion and/or deletion, which variant has a catalytic core domain, in which the variant

- at position 5 holds an alanine residue (A), a serine residue (S), or a threonine residue (T);
- at position 6 holds a threonine residue (T);
- at position 7 holds an arginine residue (R);
- at position 8 holds a phenylalanine residue (F), or a tyrosine residue (Y);
- at position 9 holds a phenylalanine residue (F), a tryptophan residue (W), or a tyrosine residue (Y);
- at position 10 holds an aspartic acid residue (D);
- at position 119 holds a holds a histidine residue (H); and
- at position 121 holds an aspartic acid residue (D) (cellulase numbering).

DETAILED DISCLOSURE OF THE INVENTION

Cellulase Variants

The present invention provides new cellulase variants derived from a parental cellulase by substitution, insertion and/or deletion. A cellulase variant of this invention is a cellulase variant or mutated cellulase, having an amino acid sequence not found in nature. The cellulase variants of the invention show improved performance, in particular with respect to increased catalytic activity; and/or altered sensitivity to anionic tensides; and/or alt red pH optimum.

Formally the cellulase variant or mutated cellulase of this inv ntion may be regarded a functional derivative of a parental cellulase (i.e. the native or wild-type nzyme), and may be obtained by alteration of a DNA nucleotide sequence of the parental gene or its derivatives, encoding the parental enzyme. The cellulase variant or mutated cellulase may be expressed and produced when the DNA nucleotide sequence encoding the cellulase variant is inserted into a suitable vector in a suitable host organism. The host organism is not necessarily identical to the organism from which the parental gene originated.

In the literature, enzyme variants have also been referred to as mutants or muteins.

Amino Acids

In the context of this invention the following symbols and abbreviations for amino acids and amino acid residues are used:

A	=	Ala	=	Alanine
C	=	Cys	=	Cysteine
D	=	Asp	=	Aspartic acid
E	=	Glu	=	Glutamic acid
F	=	Phe	=	Phenylalanine
G	=	Gly	=	Glycine
H	=	His	=	Histidine
I	=	Ile	=	Isoleucine
K	=	Lys	=	Lysine
L	=	Leu	=	Leucine
M	=	Met	=	Methionine
N	=	Asn	=	Asparagine
P	=	Pro	=	Proline
Q	=	Gln	=	Glutamine
R	=	Arg	=	Arginine
S	=	Ser	=	Serine
T	=	Thr	=	Threonine
V	=	Val	=	Valine
W	=	Trp	=	Tryptophan
Y	=	Tyr	=	Tyrosine
B	=	Asx	=	Asp or Asn
Z	=	Glx	=	Glu or Gln
X	=	Xaa	=	Any amino acid
*	=	Del tion or absent amino acid		

Cellulase Numbering

In the context of this invention a specific numbering of amino acid residue positions in cellulolytic enzymes is employed. By aligning the amino acid sequences of known cellulases, as in Table 1 below, it is possible to unambiguously allot an amino acid position number to any amino acid residue in any cellulolytic enzyme, if its amino acid sequence is known.

In Table 1, below, 11 selected amino acid sequences of cellulases of different microbial origin are aligned. These are (a) *Humicola insolens*; (b) *Acremonium sp.*; (c) *Volutelia colletotrichoides*; (d) *Sodaria fimicola*; (e) *Thielavia terrestris*; (f) *Fusarium oxysporum*; (g) *Myceliopthora thermophilia*; (h) *Crinipellis scabella*; (i) *Macrophomina phaseolina*; (j) *Pseudomonas fluorescens*; (k) *Ustilago maydis*. The cellulases (a - i) are described in PCT/DK96/00105, (j) is described in GeneBank under the accession number G45498, and (k) is described in GeneBank under the accession number S81598 and in Biol. Chem. Hoppe-Seyler 1995 **376** (10) 617-625.

Using the numbering system originating from the amino acid sequence of the cellulase obtained from a strain of *Humicola insolens* (cf. e.g. WO 91/17243), which sequence is shown in the first column of Table 1, aligned with the amino acid sequence of a number of other cellulases, it is possible to indicate the position of an amino acid residue in a cellulolytic enzyme unambiguously.

In describing the various cellulase variants produced or contemplated according to the invention, the following nomenclatures are adapted for ease of reference:

[Original amino acid; Position; Substituted amino acid]

Accordingly, the substitution of glutamine with histidine in position 119 is designated as Q119H.

Amino acid residues which represent insertions in relation to the amino acid sequence of the cellulase from *Humicola insolens*, are numbered by the addition of letters in alphabetical order to the preceding cellulase number, such as e.g. position *21aV for the "inserted" valine (V), where no amino acid residue is present, between lysine at position 21 and alanine at position 22 of the amino acid sequence of the cellulase from *Humicola insolens*, cf. Table 1.

Deletion of a proline (P) at position 49 in the amino acid sequence of the cellulase from *Humicola insolens* is indicated as P49*.

Multiple mutations are separated by slash marks ("/"), e.g.

Q119H/Q146R, representing mutations in positions 119 and 146 substituting glutamine (Q) with histidine (H), and glutamine (Q) acid with arginine (R), respectively.

If a substitution is made by mutation in e.g. a cellulase derived from a strain of *Humicola insolens*, the product is designated e.g. "*Humicola insolens*/*49P".

All positions referred to in this application by cellulase numbering refer to the cellulase numbers described above, and are determined relative to the amino acid sequence of the cellulase derived from *Humicola insolens*, cf. Table 1, (a).

Table 1

Amino Acid Sequence Alignment;

Cellulase Numbering of Selected Cellulases of Different Microbial Origin

(a) *Humicola insolens*; (b) *Acremonium sp.*; (c) *Volutelia colletotrichoides*; (d) *Sodaria fimicola*; (e) *Thielavia terrestris*; (f) *Fusarium oxysporum*; (g) *Myceliopthora thermophilia*; (h) *Crinipellis scabella*; (i) *Macrophomina phaseolina*; (j) *Pseudomonas fluorescens*; (k) *Ustilago maydis*.

	a	b	c	d	e	f	g	h	i	j	k
1	A	G	G	G	G	G	G	T	T	C	*
2	D	S	T	S	S	S	I	A	S	N	*
3	G	G	G	G	G	G	G	G	G	G	G
4	R	H	R	K	Q	H	Q	V	V	Y	M
5	S	T	T	S	S	S	T	T	T	A	A
6	T	T	T	T	T	T	T	T	T	T	T
7	R	R	R	R	R	R	R	R	R	R	R
8	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
9	W	W	W	W	W	W	W	W	W	W	W
10	D	D	D	D	D	D	D	D	D	D	D
11	C	C	C	C	C	C	C	C	C	C	C
12	C	C	C	C	C	C	C	C	C	C	C
13	K	K	K	K	K	K	K	K	K	K	L
14	P	P	P	P	P	P	P	P	P	P	A
15	S	S	S	S	S	S	S	S	S	H	S
16	C	C	C	C	C	C	C	C	C	C	A
17	G	A	G	A	A	S	A	G	A	G	S
18	W	W	W	W	W	W	W	W	W	W	W

19	A	D	D	S	P	S	P	S	T	S	E
20	K	E	E	G	G	G	G	G	G	A	G
21	K	K	K	K	K	K	K	K	K	N	K
21a	*	*	*	*	*	*	*	*	*	V	*
22	A	A	A	A	A	A	G	A	A	P	A
23	P	A	S	S	A	A	P	S	S	S	P
24	V	V	V	V	V	V	*	V	V	L	V
25	N	S	S	N	S	N	S	S	S	V	Y
26	Q	R	Q	R	Q	A	S	A	K	S	A
27	P	P	P	P	P	P	P	P	P	P	P
28	V	V	V	V	V	A	V	V	V	L	V
29	F	T	K	L	Y	L	Q	R	G	Q	D
30	S	T	T	A	A	T	A	T	T	S	A
31	C	C	C	C	C	C	C	C	C	C	C
32	N	D	D	D	D	D	D	D	D	S	K
33	A	R	R	A	A	K	K	R	I	A	A
34	N	N	N	N	N	N	N	N	N	N	D
35	F	N	N	N	F	D	D	G	D	N	G
36	Q	S	N	N	Q	N	N	N	N	T	V
37	R	P	P	P	R	P	P	T	A	R	T
38	I	L	L	L	L	I	F	L	Q	L	L
39	T	S	A	N	S	S	N	G	T	S	I
40	D	P	S	D	D	N	D	P	P	D	D
41	F	*	*	A	F	T	G	*	S	V	S
42	D	G	T	N	N	N	G	*	D	S	K
42a	*	*	*	*	*	*	S	D	L	*	K
43	A	A	A	V	V	A	T	V	L	V	D
44	K	V	R	K	Q	V	R	K	K	G	P
45	S	S	S	S	S	N	S	S	S	S	S
46	G	G	G	G	G	G	G	G	S	S	G
47	C	C	C	C	C	C	C	C	C	C	Q
48	E	D	D	D	N	E	D	D	D	D	S
49	P	P	S	*	*	G	A	S	*	*	G
49a	*	*	*	*	*	*	*	*	*	*	C
49b	*	*	*	*	*	*	*	*	*	*	N
50	G	N	N	G	G	G	G	G	G	G	G
51	G	G	G	G	G	G	G	G	G	G	G
52	V	V	V	S	S	S	S	T	S	G	N
53	A	A	A	A	A	A	A	S	A	G	K
54	Y	F	Y	Y	Y	Y	Y	F	Y	Y	F
55	S	T	T	T	S	A	M	T	Y	M	M
56	C	C	C	C	C	C	C	C	C	C	C
57	A	N	N	A	A	T	S	A	S	W	S
58	D	D	D	N	D	N	S	N	N	D	C
59	Q	N	N	N	Q	Y	Q	N	Q	K	M
60	T	Q	Q	S	T	S	S	G	G	I	Q
61	P	P	P	P	P	P	P	P	P	P	P
62	W	W	W	W	W	W	W	F	W	F	F

63	A	A	A	A	A	A	A	A	A	A	D
64	V	V	V	V	V	V	V	I	V	V	D
65	N	N	N	N	N	N	S	D	N	S	E
66	D	N	D	D	D	D	D	N	D	P	T
67	D	N	N	N	N	E	E	N	S	T	D
68	F	V	L	L	L	L	L	T	L	L	P
69	A	A	A	A	A	A	S	A	S	A	T
70	L	Y	Y	Y	Y	Y	Y	Y	Y	Y	L
71	G	G	G	G	G	G	G	G	G	G	A
72	F	F	F	F	F	F	W	F	F	Y	F
73	A	A	A	A	A	A	A	A	A	A	G
74	A	A	A	A	A	A	A	A	A	A	F
75	T	T	T	T	T	T	V	A	A	T	G
76	S	A	A	K	S	K	K	H	K	S	A
77	I	F	F	L	I	I	L	L	L	S	F
78	A	P	S	S	A	S	A	A	S	G	T
79	G	G	G	G	G	G	G	G	G	D	T
80	S	G	G	G	G	G	S	S	K	V	G
81	N	N	S	T	S	S	S	S	Q	*	Q
82	E	E	E	E	E	E	E	E	E	*	E
83	A	A	A	S	S	A	S	A	T	*	S
84	G	S	S	S	S	S	Q	A	D	*	D
85	W	W	W	W	W	W	W	W	W	*	T
86	C	C	C	C	C	C	C	C	C	C	D
87	C	C	C	C	C	C	C	C	C	G	C
88	A	A	A	A	A	A	A	Q	G	R	A
89	C	C	C	C	C	C	C	C	C	C	C
90	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	F
91	E	A	A	A	A	A	E	E	K	Q	Y
92	L	L	L	L	L	L	L	L	L	L	A
93	T	Q	Q	T	T	T	T	T	T	Q	E
94	F	F	F	F	F	F	F	F	F	F	F
95	T	T	T	T	T	T	T	T	T	T	E
95a	*	*	*	*	*	*	*	*	*	G	*
95b	*	*	*	*	*	*	*	*	*	S	*
95c	*	*	*	*	*	*	*	*	*	S	*
95d	*	*	*	*	*	*	*	*	*	Y	*
95e	*	*	*	*	*	*	*	*	*	N	*
95f	*	*	*	*	*	*	*	*	*	A	*
95g	*	*	*	*	*	*	*	*	*	P	*
95h	*	*	*	*	*	*	*	*	*	G	H
95i	*	*	*	*	*	*	*	*	*	D	D
95j	*	*	*	*	*	*	*	*	*	P	A
95k	*	*	*	*	*	*	*	*	*	G	Q
96	S	S	S	S	S	T	S	S	S	S	G
97	G	G	G	G	G	G	G	G	T	A	K
98	P	P	P	P	P	P	P	P	A	A	A
99	V	V	V	V	V	V	V	V	V	L	M

100	A	A	A	S	A	K	A	V	S	A	K
101	G	G	G	G	G	G	G	G	G	G	R
102	K	K	K	K	K	K	K	K	K	K	N
103	K	T	T	T	T	K	K	K	Q	T	K
104	M	M	M	L	M	M	M	L	M	M	L
105	V	V	V	V	V	I	I	T	I	I	I
106	V	V	V	V	V	V	V	V	V	V	F
107	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
108	S	S	S	S	S	S	A	V	I	A	V
109	T	T	T	T	T	T	T	T	T	T	T
110	S	N	N	S	S	N	N	N	N	N	N
111	T	T	T	T	T	T	T	T	T	I	V
112	G	G	G	G	G	G	G	G	G	G	G
113	G	G	G	G	G	G	G	G	G	Y	G
114	D	D	D	D	D	D	D	D	D	D	D
115	L	L	L	L	L	L	L	L	L	V	V
116	G	S	S	G	G	G	G	G	G	S	Q
117	S	G	G	S	S	D	D	N	N	G	S
118	N	T	N	N	N	N	N	N	N	G	Q
119	H	H	H	H	Q	H	H	H	H	Q	N
120	F	F	F	F	F	F	F	F	F	F	F
121	D	D	D	D	D	D	D	D	D	D	D
122	L	I	I	L	I	L	L	L	I	I	F
123	N	Q	L	N	A	M	A	M	A	L	Q
124	I	M	M	M	M	M	I	I	M	V	I
125	P	P	P	P	P	P	P	P	P	P	P
126	G	G	G	G	G	G	G	G	G	G	G
127	G	G	G	G	G	G	G	G	G	G	G
128	G	G	G	G	G	G	G	G	G	G	G
129	V	L	L	V	V	V	V	V	V	V	L
130	G	G	G	G	G	G	G	G	G	G	G
131	I	I	I	L	I	I	I	L	I	A	A
132	F	F	F	F	F	F	F	F	F	F	F
132a	*	*	*	*	*	*	*	T	*	*	P
133	D	D	D	D	N	D	N	Q	N	N	K
134	G	G	G	G	G	G	A	G	G	A	G
135	C	C	C	C	C	C	C	C	C	C	C
136	T	T	T	K	S	T	T	P	S	S	P
137	P	P	P	R	S	S	D	A	K	A	A
138	Q	Q	Q	E	Q	E	Q	Q	Q	Q	Q
139	F	F	W	F	F	F	Y	F	W	W	W
140	G	G	G	G	G	G	G	G	N	G	G
140a	*	F	V	*	*	K	A	S	G	V	V
141	G	T	S	G	G	A	P	W	I	S	E
142	L	F	F	L	L	L	P	N	*	N	A
143	P	P	P	P	P	G	N	G	*	A	S
143a	*	*	*	*	*	*	G	*	N	E	L
143b	*	*	*	*	*	*	W	*	L	L	W

144	G	G	G	G	G	G	G	G	G	G	G
145	Q	N	N	A	A	A	D	A	N	A	D
146	R	R	R	Q	Q	Q	R	Q	Q	Q	Q
147	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
148	G	G	G	G	G	G	G	G	G	G	G
149	G	G	G	G	G	G	G	G	G	G	G
150	I	T	T	I	I	I	I	V	F	F	V
150a	*	*	*	*	*	*	*	*	*	L	*
150b	*	*	*	*	*	*	*	*	*	A	*
150c	*	*	*	*	*	*	*	*	*	A	*
150d	*	*	*	*	*	*	*	*	*	C	*
150e	*	*	*	*	*	*	*	*	*	K	*
150f	*	*	*	*	*	*	*	*	*	Q	*
150g	*	*	*	*	*	*	*	*	*	Q	*
150h	*	*	*	*	*	*	*	*	*	L	*
150i	*	*	*	*	*	*	*	*	*	G	*
150j	*	*	*	*	*	*	*	*	*	Y	*
150k	*	*	*	*	*	*	*	*	*	N	*
151	S	T	T	S	S	S	H	S	T	A	K
152	S	S	S	S	S	S	S	S	D	S	S
153	R	R	R	R	R	R	K	R	R	L	A
154	N	S	S	S	D	S	E	D	S	S	T
155	E	Q	Q	E	Q	E	E	Q	Q	Q	E
156	C	C	C	C	C	C	C	C	C	Y	C
157	D	A	S	D	D	D	E	S	A	K	S
158	R	E	Q	S	S	S	S	Q	T	S	K
159	F	L	I	F	F	Y	F	L	L	C	L
160	P	P	P	P	P	P	P	P	P	V	P
160a	*	*	*	*	*	*	*	*	*	L	*
160b	*	*	*	*	*	*	*	*	*	N	*
160c	*	*	*	*	*	*	*	*	*	R	*
160d	*	*	*	*	*	*	*	*	*	C	*
160e	*	*	*	*	*	*	*	*	*	D	*
160f	*	*	*	*	*	*	*	*	*	S	*
160g	*	*	*	*	*	*	*	*	*	V	*
160h	*	*	*	*	*	*	*	*	*	F	*
160i	*	*	*	*	*	*	*	*	*	G	*
160j	*	*	*	*	*	*	*	*	*	S	*
160k	*	*	*	*	*	*	*	*	*	R	*
160l	*	*	*	*	*	*	*	*	*	G	*
160m	*	*	*	*	*	*	*	*	*	L	*
161	D	S	S	A	A	E	E	A	S	T	K
162	A	V	A	A	P	L	A	A	K	Q	P
163	L	L	L	L	L	L	L	V	W	L	L
164	K	R	Q	K	K	K	K	Q	Q	Q	Q
165	P	D	P	P	P	D	P	A	A	Q	E
166	G	G	G	G	G	G	G	G	S	G	G
167	C	C	C	C	C	C	C	C	C	C	C

168	Y	H	N	Q	Q	H	N	Q	N	T	K
169	W	W	W	W	W	W	W	F	W	W	W
170	R	R	R	R	R	R	R	R	R	F	R
171	F	Y	Y	F	F	F	F	F	F	A	F
172	D	D	D	D	D	D	D	D	D	E	S
173	W	W	W	W	W	W	W	W	W	W	E
174	F	F	F	F	F	F	F	M	F	F	W
175	K	N	N	K	Q	E	Q	G	E	E	G
176	N	D	D	N	N	N	N	G	N	A	D
177	A	A	A	A	A	A	A	A	A	A	N
178	D	D	D	D	D	D	D	D	D	D	P
179	N	N	N	N	N	N	N	N	N	N	V
180	P	P	P	P	P	P	P	P	P	P	L
181	S	N	D	E	T	D	S	N	T	S	K
182	F	V	V	F	F	F	V	V	V	L	G
183	S	N	S	T	T	T	T	T	D	K	S
184	F	W	W	F	F	F	F	F	W	Y	P
185	R	R	R	K	Q	E	Q	R	E	K	K
186	Q	R	R	Q	Q	Q	E	P	P	E	R
187	V	V	V	V	V	V	V	V	V	V	V
188	Q	R	Q	Q	Q	Q	A	T	T	P	K
189	C	C	C	C	C	C	C	C	C	C	C
190	P	P	P	P	P	P	P	P	P	P	P
191	A	A	A	S	A	K	S	A	Q	A	K
192	E	A	A	E	E	A	E	Q	E	E	S
193	L	L	L	L	I	L	L	L	L	L	L
194	V	T	T	T	V	L	T	T	V	T	I
195	A	N	D	S	A	D	S	N	A	T	D
196	R	R	R	R	R	I	K	I	R	R	R
197	T	S	T	T	S	S	S	S	T	S	S
198	G	G	G	G	G	G	G	G	G	G	G
199	C	C	C	C	C	C	C	C	C	M	C
200	R	V	R	K	K	K	S	V	S	N	Q
201	R	R	R	R	R	R	R	R	R	R	R

* Amino acid residue absent in this position

Family 45 Endo-glucanases

The present invention relates to cellulase variants. More specifically the pr sent invention provides cellulase variant derived from a parental cellulase by substitution, insertion and/or deletion, which variant has a catalytic core domain, in which the variant

at position 5 holds an alanine residue (A), a serine r sidue (S), or a threonine residue (T);

- at position 6 holds a threonine residue (T);
- at position 7 holds an arginine residue (R);
- at position 8 holds a phenylalanine residue (F), or a tyrosine residue (Y);
- at position 9 holds a phenylalanine residue (F), a tryptophan residue (W), or a tyrosine residue (Y);
- at position 10 holds an aspartic acid residue (D);
- at position 119 holds a holds a histidine residue (H); and
- at position 121 holds an aspartic acid residue (D) (cellulase numbering).

The endoglucanase of the invention may comprise a cellulose binding domain (CBD) existing as an integral part of the enzyme, or a CBD from another origin may be introduced into the endoglucanase thus creating an enzyme hybride. In this context, the term "cellulose-binding domain" is intended to be understood as defined by Peter Tomme et al. "Cellulose-Binding Domains: Classification and Properties" in "Enzymatic Degradation of Insoluble Carbohydrates", John N. Saddler and Michael H. Penner (Eds.), ACS Symposium Series, No. 618, 1996. This definition classifies more than 120 cellulose-binding domains into 10 families (I-X), and demonstrates that CBDs are found in various enzymes such as cellulases, xylanases, mannanases, arabinofuranosidases, acetyl esterases and chitinases. CBDs have also been found in algae, e.g. the red alga *Porphyra purpurea* as a non-hydrolytic polysaccharide-binding protein, see Tomme et al., *op.cit.* However, most of the CBDs are from cellulases and xylanases, CBDs are found at the N and C termini of proteins or are internal. Enzyme hybrids are known in the art, see e.g. WO 90/00609 and WO 95/16782, and may be prepared by transforming into a host cell a DNA construct comprising at least a fragment of DNA encoding the cellulose-binding domain ligated, with or without a linker, to a DNA sequence encoding the endoglucanase and growing the host cell to xpress the fused gene.

Enzyme hybrids may be described by the following formula:

CBD - MR - X or X-MR-CBD

wherein CBD is the N-t rminal or the C-terminal region of an amino acid sequence corresponding to at least the cellulose-binding domain; MR is the middle

region (the linker), and may be a bond, or a short linking group preferably of from about 2 to about 100 carbon atoms, mor pr ferably of from 2 to 40 carbon atoms; or is preferably from about 2 to about 100 amino acids, more preferably of from 2 to 40 amino acids; and X is an N-terminal or C-terminal region of the enzyme according to the invention.

Disulfide Bridges

Disulfide bridges (i.e. Cys-Cys bridges) stabilize the structure of the nzyme. It is believed that a certain number of stabilizing disulfide bridges are necessary to maintain the a proper stability of the enzyme.

Therefore, in another aspect, the invention provides a cellulase variant which variant holds 4 or more of the following disulfide bridges: C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering). In a more specific embodiment the variant of the invention holds 5 or more of the following disulfide bridges: C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering). In its most specific embodiment, the variant of the invention holds 6 or more of the following disulfide bridges: C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering).

In another embodiment the invention provides a cellulase variant in which cysteine has been replaced by another natural amino acid at one or more of the positions 16, 86, 87, 89, 189, and/or 199 (cellulase numbering).

Binding Cleft Substitutions

In a third aspect, the invention provides a cellulase variant derived from a parental cellulase by substitution, insertion and/or deletion at one or more amino acid residues located in the substrate binding cleft. Mutations introduced at positions close to the substrate affect the enzyme-substrate interactive bindings.

An appropriate way of determining the residues interacting with a potential substrate in a structure is to partitionate th structur in "sh lls". The shells are defined as: 1st shell are residues directly interacting with the substrate, *i.e.* closest inter atomic distance betwe n substrate and residue both including hydrogen atoms

are smaller than 2.5Å which will include all direct interaction via hydrogen bonds and other non bonded interactions. The subsequent (2^{nd},3^{rd} .t.c.) shells are defined in the same way, as the residues with inter atomic distances smaller than 2.5Å to the substrate or all previously determined shells. In this way the structure will be partitioned in shells. The routine "*subset zone*" in the program *Insight II 95.0* (*Insight II 95.0 User Guide*, October 1995. San Diego: Biosym/MSI, 1995.) can be used to determine the shells.

In a preferred embodiment, the amino acid residue contemplated according to this invention is located in the substrate binding cleft at a distance of up to 5 Å from the substrate.

When subjecting the *Humicola insolens* family 45 cellulase to computer modeling according to Example 1, below, and aligning these positions to other family 45 cellulases, the following positions, which are within a distance of up to 5 Å from the substrate, are revealed: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 21a, 42, 44, 45, 47, 48, 49, 49a, 49b, 74, 82, 95j, 110, 111, 112, 113, 114, 115, 116, 119, 121, 123, 127, 128, 129, 130, 131, 132, 132a, 133, 145, 146, 147, 148, 149, 150b, 178, and/or 179 (cellulase numbering), cf. Table 2.

Accordingly, in a more specific embodiment, the invention provides a cellulase variant which has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of these acid residues. In a particular embodiment, the cellulase variant is derived from one of the cellulases identified in Table 2 ((a) *Humicola insolens*; (b) *Acremonium sp.*; (c) *Volutelia colletotrichoides*; (d) *Sodaria fimicola*; (e) *Thielavia terrestris*; (f) *Fusarium oxysporum*; (g) *Myceliopthora thermophilia*; (h) *Crinipellis scabella*; (i) *Macrophomina phaseolina*; (j) *Pseudomonas fluorescens*; (k) *Ustilago maydis*), by substitution, insertion and/or deletion at one or more of the positions identified in Table 2 for these cellulases.

Table 2

Amino Acid Residues less than 5 Å from the Substrate

Positions Identified by Cellulase Numbering

(a) *Humicola insolens*; (b) *Acremonium sp.*; (c) *Volutelia colletotrichoides*; (d) *Sodaria fimicola*; ( ) *Thielavia terrestris*; (f) *Fusarium oxysporum*; (g) *Myceliopthora thermophilia*; (h) *Crinipellis scabella*; (i) *Macrophomina phaseolina*; (j) *Pseudomonas fluorescens*; (k) *Ustilago maydis*.

	a	b	c	d	e	f	g	h	i	j	k
4										Y	
5	S	T	T	S	S	S	T	T	T	A	A
6	T	T	T	T	T	T	T	T	T	T	T
7	R	R	R	R	R	R	R	R	R	R	R
8	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
9	W	W	W	W	W	W	W	W	W	W	W
10	D	D	D	D	D	D	D	D	D	D	D
11							C				
12	C	C	C	C	C	C	C	C	C	C	C
13	K	K	K	K	K	K	K	K	K	K	L
14	P	P	P	P	P	P	P	P	P	P	A
15	S	S	S	S	S	S	S	S	S	H	S
16										C	
18	W	W	W	W	W	W	W	W	W	W	W
19	A	D	D	S	P	S	P	S	T		E
20	K	E	E		G		G	G		A	
21	K	K	K	K	K	K	K	K	K		K
21a										V	
42		G	T						D		
44		V	R	K	Q	V	R			G	
45	S	S	S	S	S	N	S	S	S	S	
47	C	C	C	C	C	C	C	C	C	C	
48	E	D	D	D	N	E	D	D	D	D	S
49		P									
49a											C
49b											N
74	A	A	A	A	A	A	A	A	A	A	F
82	E	E	E	E	E	E		E	E		E
95j										P	
110	S	N	N	S	S	N	N	N	N	N	N
111	T	T	T	T	T	T	T	T	T	I	V
112	G	G	G	G	G	G	G	G	G	G	G
113	G	G	G	G	G	G	G	G	G	Y	G
114	D	D	D	D	D	D	D	D	D	D	D
115	L	L	L	L	L	L	L	L	L	V	V
116					G						
119	H	H	H	H	Q	H	H	H	H	Q	N

121	D	D	D	D	D	D	D	D	D	D	D
123		Q									
127	G	G	G	G	G	G	G	G	G	G	G
128	G	G	G	G	G	G	G	G	G	G	G
129	V	L	L	V	V	V	V	V	V	V	L
130	G	G	G	G	G	G	G	G	G	G	G
131	I	I	I	L	I	I	I	L	I	A	A
132	F	F	F	F	F	F	F	F	F	F	F
132a								T			P
133									N	N	
145										A	D
146	R	R	R	Q	Q	Q	R	Q	Q	Q	Q
147	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
148	G	G	G	G	G	G	G	G	G	G	G
149	G			G	G	G			G		
150b										A	
178	D	D	D	D	D	D	D	D	D	D	P
179	N	N	N	N	N	N	N	N	N	N	V

In another preferred embodiment, the amino acid residue contemplated according to this invention is located in the substrate binding cleft at a distance of up to 3 Å from the substrate.

When subjecting the *Humicola insolens* family 45 cellulase to computer modeling according to Example 1, below, and aligning these positions to other family 45 cellulases, the following positions, which are within a distance of up to 3 Å from the substrate, are revealed: 6, 7, 8, 10, 12, 13, 14, 15, 18, 20, 21, 45, 48, 74, 110, 111, 112, 113, 114, 115, 119, 121, 127, 128, 129, 130, 131, 132, 132a, 146, 147, 148, 150b, 178, and/or 179 (cellulase numbering). cf. Table 3.

Accordingly, in a more specific embodiment, the invention provides a cellulase variant which has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of these acid residues. In a particular embodiment, the cellulase variant is derived from one of the cellulases identified in Table 3 ((a) *Humicola insolens*; (b) *Acremonium sp.*; (c) *Volutelia colletotrichoides*; (d) *Sodaria fimicola*; (e) *Thielavia terrestris*; (f) *Fusarium oxysporum*; (g) *Myceliopthora thermophilia*; (h) *Crinipellis scabella*; (i) *Macrophomina phaseolina*; (j) *Pseudomonas fluorescens*; (k) *Ustilago maydis*), by substitution, insertion and/or deletion at one or more of the positions id ntified in Table 3 for these cellulases.

Table 3

Amino Acid Residues less than 3 Å from the Substrate

Positions Identified by Cellulase Numbering

(a) *Humicola insolens*; (b) *Acremonium sp.*; (c) *Volutelia colletotrichoides*; (d) *Sodaria fimicola*; (e) *Thielavia terrestris*; (f) *Fusarium oxysporum*; (g) *Myceliopthora thermophilia*; (h) *Crinipellis scabella*; (i) *Macrophomina phaseolina*; (j) *Pseudomonas fluorescens*; (k) *Ustilago maydis*.

	a	b	c	d	e	f	g	h	i	j	k
6	T	T	T	T	T	T	T	T	T	T	T
7	R	R	R	R	R	R	R	R	R	R	R
8	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10	D	D	D	D	D	D	D	D		D	D
12	C	C	C	C	C	C	C	C	C	C	C
13	K	K	K	K		K			K		L
14		P			P	P	P	P	P		A
15	S	S	S	S	S	S	S	S	S	H	S
18	W	W	W	W	W	W	W	W	W	W	W
20		E	E								
21	K			K							
45	S	S	S	S	S	N	S	S	S	S	S
48		D			N	E	D			D	
74	A			A	A	A	A	A	A		F
110		N	N		S	N	N	N	N	N	N
111	T	T	T	T	T	T	T	T	T		
112	G	G	G	G	G	G	G	G	G	G	G
113	G	G	G	G				G	G	Y	G
114	D	D	D	D	D	D	D	D	D	D	D
115	L	L	L	L	L	L	L	L	L	V	V
119	H	H	H		Q	H		H		Q	
121	D	D	D	D	D	D	D	D	D	D	D
127	G			G	G	G	G		G		
128	G			G	G	G	G	G	G		G
129	V	L	L	V	V	V	V	V	V	V	L
130	G	G	G	G	G	G	G	G	G	G	G
131	I	I	I	L	I	I	I	L	I	A	A
132	F	F	F	F	F	F	F	F	F	F	F
132a								T			P
146					Q			Q		Q	Q

147	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
148	G	G	G	G	G	G	G	G	G	G	G
150b										A	
178	D	D	D	D				D		D	P
179	N	N	N	N	N	N	N	N	N	N	

Conserved Amino Acid Residues

As defined herein a conserved amino acid residue is an amino acid residue identified according to Table 1, at a position at which position between 7 to 10 amino acid residues of the 11 residues indicated in Table 1 for that position, are identical.

Therefore, in its fourth aspect, the invention provides a cellulase variant, in which variant an amino acid residue has been changed into a conserved amino acid residue at one or more positions according to Table 1, at which position(s) between 7 and 10 amino acid residues of the 11 residues identified in Table 1, are identical.

In a preferred embodiment the invention provides a cellulase variant, which has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 13, 14, 15, 20, 21, 22, 24, 28, 32, 34, 45, 48, 50, 53, 54, 62, 63, 64, 65, 66, 68, 69, 70, 71, 72, 73, 74, 75, 79, 85, 88, 90, 92, 93, 95, 96, 97, 98, 99, 104, 106, 110, 111, 113, 115, 116, 118, 119, 131, 134, 138, 140, 146, 152, 153, 163, 166, 169, 170, 171, 172, 173, 174, 174, 177, 178,179, 180, 193, 196, and/or 197 (cellulase numbering).

In a more specific embodiment the invention provides a cellulase variant that has been subjected to substitutions, insertions and/or deletions, so as to comprise one or more of the amino acid residues at the positions identified in Table 4, below.

Table 4

Selected Substitutions, Insertions and/or Deletions

Positions Identified by Cellulase Numbering

Position	Amino Acid Residue
4	R,H,K,Q,V,Y,M

5	S,T,A
13	K,L
14	P,A
15	H,S
16	C,A
19	A,D,S,P,T,E
20	A, E,G,K
21	K, N
21a	V,*
22	A, G, P
24	*, L, V
28	A, L, V
32	D, K, N, S
34	D, N
38	F, I, L, Q
42	D,G,T,N,S,K,*
44	K,V,R,Q,G,P
45	N, S
46	G, S
47	C,Q
48	D,E,N,S
49	P,S,A,G,*
49a	C,*
49b	N,*
50	G, N
53	A, G, K, S
54	F, Y
62	F, W
63	A, D
64	D, I V
65	D, E, N, S
66	D, N, P, T
68	F, L, P, T, V
69	A, S, T
70	L,Y
71	A,G
72	F,W,Y
73	A,G
74	A,F
75	A,G,T,V
79	G, T
82	E,*
88	A, G, Q, R
90	F, Y
92	A,L
93	E,Q,T
95	E,T
95j	P,*

96	S,T
97	A,G,T
98	A,P
99	L,V
104	L,M
106	F,V
110	N,S
111	I,T,V
113	G,Y
115	L,V
116	G,Q,S
118	G,N,Q,T
119	H,N,Q
129	L,V
131	A,I,L
132	A,P,T,*
133	D,K,N,Q
134	A, G
138	E, Q
145	A,D,N,Q
146	Q,R
150b	A,*
152	D,S
153	A,K,L,R
163	L,V,W
166	G,S
169	F,W
170	F,R
171	A,F,Y
172	D,E,S
173	E,W
174	F,M,W
177	A,N
178	D,P
179	N,V
180	L,P
193	I,L
196	I,K,R
197	S,T

In a yet more preferred embodiment, the invention provides a cellulase variant derived from a parental cellulase by substitution, insertion and/or deletion at one or more amino acid residues as indicated in Tables 5-6, below. The cellulase variant may be derived from any parental cellulase holding the amino acid residue

stated at the position indicated. In particular the parental cellulas may be a *Humicola insolens* cellulase; an *Acremonium sp.* Cellulase; a *Volutelia colletotrichoides* cellulase; a *Sodaria fimicola* cellulase; a *Thielavia terrestris* cellulase; a *Fusarium oxysporum* cellulase; a *Myceliopthora thermophilia* cellulase; a *Crinipellis scabella* cellulase; a *Macrophomina phaseolina* cellulase; a *Pseudomonas fluorescens* cellulase; or a *Ustilago maydis* cellulase.

Moreover, the cellulase variant may be characterized by having improved performance, in particular with respect to

1) improved performance defined as increased catalytic activity;

2) altered sensitivity to anionic tenside; and/or

3) altered pH optimum;

as also indicated in the tables.

Table 5

Preferred Cellulase Variants

Positions Identified by Cellulase Numbering

K13L, L13K (1,2,3);

P14A, A14P (1);

S15H, H15S (1,3);

K20E, K20G, K20A, E20K, G20K, A20K, E20G, E20A, G20E, A20E, G20A, A20G (1,2,3);

K21N, N21K (1,2,3);

A22G, A22P, G22A, P22A, G22P, P22G (1);

V24*, V24L, *24V, L24V, *24L, L24* (1);

V28A, V28L, A28V, L28V, A28L, L28A (1);

N32D, N32S, N32K, D32N, S32N, K32N, D32S, D32K, S32D, K32D, S32K, K32S (2,3);

N34D, D34N (2);

I38L, I38F, I38Q, L38I, F38I, Q38I, L38F, L38Q, F38L, Q38L, F38Q, Q38F (1)

S45N, N45S (1);

G46S, S46G (1);

E48D, E48N, D48E, N48E, D48N, N48D (1,2,3);

G50N, N50G (1);

A53S, A53G, A53K, S53A, G53A, K53A, S53G, S53K, G53S, K53S, G53K, K53G (1);

Y54F, F54Y (1,3);

W62F, F62W (1,2);

A63D, D63A (2,3);

V64I, V64D, I64V, D64V, I64D, D64I (2);

N65S, N65D, N65E, S65N, D65N, E65N, S65D, S65E, D65S, E65S, D65E, E65D (2);

D66N, D66P, D66T, N66D, P66D, T66D, N66P, N66T, P66N, T66N, P66T, T66P (2,3);

F68V, F68L, F68T, F68P, V68F, L68F, T68F, P68F, V68L, V68T, V68P, L68V, T68V, P68V, L68T, L68P, T68L, P68L, T68P, P68T (1,2);

A69S, A69T, S69A, T69A, S69T, T69S (1);

L70Y, Y70L (1);

G71A, A71G (1);

F72W, F72Y, W72F, Y72F, W72Y, Y72W (1);

A73G, G73A (1);

A74F, F74A (1);

T75V, T75A, T75G, V75T, A75T, G75T, V75A, V75G, A75V, G75V, A75G, G75A (1);

G79T, T79G (1);

W85T, T85W (1);

A88Q, A88G, A88R, Q88A, G88A, R88A, Q88G, Q88R, G88Q, R88Q, G88R, R88G (1,2,3);

Y90F, F90Y (1);

L92A, A92L (1);

T93Q, T93E, Q93T, E93T, Q93E, E93Q (2);

T95E, E95T (2);

S96T, T96S (1);

G97T, G97A, T97G, A97G, T97A, A97T (1);

P98A, A98P (1);

V99L, L99V (1);

M104L, L104M (1);

V106F, F106V (1,3);

S110N, N110S (1);

T111I, T111V, I111T, V111T, I111V, V111I (1);

G113Y, Y113G (1,3);

L115V, V115L (1);

G116S, G116Q, S116G, Q116G, S116Q, Q116S (1);

N118T, N118G, N118Q, T118N, G118N, Q118N, T118G, T118Q, G118T, Q118T, G118Q, Q118G (1);

H119Q, H119N, Q119H, N119H (1,2);

V129L, L129V (1);

I131L, I131A, L131I, A131I, L131A, A131L (1);

G134A, A134G (1);

Q138E, E138Q (1,2,3);

G140N, N140G (1);

R146Q, Q146R (1,2,3);

S152D, D152S (2);

R153K, R153L, R153A, K153R, L153R, A153R, K153L, K153A, L153K, A153K, L153A, A153L (2);

L163V, L163W, V163L, W163L, V163W, W163V (1);

G166S, S166G (1);

W169F, F169W (1);

R170F, F170R (1,2,3);

F171Y, F171A, Y171F, A171F, Y171A, A171Y (1);

D172E, D172S, E172D, S172D, E172S, S172E (2);

W173E, E173W (1,2,3);

F174M, F174W, M174F, W174F, M174W, W174M (1);

A177N, N177A (1);

D178P, P178D (1,2,3);

N179V, V179N (1);

P180L, L180P (1);

L193I, I193L (1);

R196I, R196K, I196R, K196R, I196K, K196I (2,3);

T197S, S197T (1)

Table 6

Preferred Cellulase Variants

Positions Identified by Cellulase Numbering

L13K (1,2,3);

A14P (1);

H15S (1,3);

E20K, G20K, A20K (1,2,3);

N21K (1,2,3);

G22A, P22A (1);

*24V, L24V (1);

A28V, L28V (1);

D32N, S32N, K32N (2,3);

D34N (2);

L38I, F38I, Q38I (1);

N45S (1);

S46G (1);

D48E, N48E (1,2,3);

N50G (1);

S53A, G53A, K53A (1);

F54Y (1,3);

F62W (1,2);

D63A (2,3);

I64V, D64V (2);

S65N, D65N, E65N (2)

N66D, P66D, T66D (2,3);

V68F, L68F, T68F, P68F (1,2);

S69A, T69A (1)

Y70L (1)
A71G (1)
W72F, Y72F (1)
G73A (1)
F74A (1)
V75T, A75T, G75T (1)
T79G (1);
T85W (1);
Q88A, G88A, R88A (1,2,3)
F90Y (1)
A92L (1)
Q93T, E93T (2);
E95T (2);
T96S (1);
T97G, A97G (1);
A98P (1);
L99V (1);
L104M (1);
F106V (1,3);
N110S (1);
I111T, V111T (1);
Y113G (1,3);
V115L (1);
S116G, Q116G (1);
T118N, G118N, Q118N (1);
Q119H, N119H (1,2);
L129V (1);
L131I, A131I (1);
A134G (1);
E138Q (1,2,3);
N140G (1);
Q146R (1,2,3);

D152S (2);
K153R, L153R, A153R (2);
V163L, W163L (1);
S166G (1);
F169W (1);
F170R (1,2,3);
Y171F, A171F (1);
E172D, S172D (2);
E173W (1,2,3);
M174F, W174F (1);
N177A (1);
P178D (1,2,3);
V179N (1);
L180P (1);
I193L (1);
I196R, K196R (2,3);
S197T (1)

Altered Sensibility Towards Anionic Tensides

Anionic tensides are products frequently incorporated into detergent compositions. Sometimes cellulolytic enzymes having an increased stability towards anionic tensides is a desire, and sometimes cellulolytic enzymes having an increased sensitivity are preferred. In its fifth aspect the invention provides cellulase variants of an altered anionic tenside sensitivity.

Accordingly, a cellulase variant of the invention of altered anionic tenside sensitivity is a cellulase variant which has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 2, 4, 7, 8, 10, 13, 15, 19, 20, 21, 25, 26, 29, 32, 33, 34, 35, 37, 40, 42, 42a, 43, 44, 48, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 70, 72, 76, 79, 80, 82, 84, 86, 88, 90, 91, 93, 95, 95d, 95h, 95j, 97, 100, 101, 102, 103, 113, 114, 117, 119, 121, 133, 136, 137, 138, 139,

140a, 141, 143a, 145, 146, 147, 150e, 150j, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160c, 160e, 160k, 161, 162, 164, 165, 168, 170, 171, 172, 173, 175, 176, 178, 181, 183, 184, 185, 186, 188, 191, 192, 195, 196, 200, and/or 201 (cellulase numbering).

In a particular embodiment, the cellulase variant is derived from one of the cellulases identified in Table 7, below, ((a) *Humicola insolens*; (b) *Acremonium sp.*; (c) *Volutelia colletotrichoides*; (d) *Sodaria fimicola*; (e) *Thielavia terrestris*; (f) *Fusarium oxysporum*; (g) *Myceliopthora thermophilia*; (h) *Crinipellis scabella*; (i) *Macrophomina phaseolina*; (j) *Pseudomonas fluorescens*; (k) *Ustilago maydis*), by substitution, insertion and/or deletion at one or more of the positions identified in Table 7 for these cellulases.

Table 7

Altered Sensitivity towards Anionic Tensides

Positions identified by Cellulase Numbering

(a) *Humicola insolens*; (b) *Acremonium sp.*; (c) *Volutelia colletotrichoides*; (d) *Sodaria fimicola*; (e) *Thielavia terrestris*; (f) *Fusarium oxysporum*; (g) *Myceliopthora thermophilia*; (h) *Crinipellis scabella*; (i) *Macrophomina phaseolina*; (j) *Pseudomonas fluorescens*; (k) *Ustilago maydis*.

	a	b	c	d	e	f	g	h	i	j	k
2	D										
4	R	H	R	K		H				Y	
7	R	R	R	R	R	R	R	R	R	R	R
8	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10	D	D	D	D	D	D	D	D	D	D	D
13	K	K	K	K	K	K	K	K	K	K	
15										H	
19		D	D								E
20	K	E	E								
21	K	K	K	K	K	K	K	K	K		K
25											Y
26		R		R					K		
29			K		Y			R			D
32		D	D	D	D	D	D	D	D		K
33		R	R			K	K	R			

34											D
35						D	D		D		
37	R				R					R	
40	D			D	D		D			D	D
42	D								D		K
42a								D			K
43											D
44	K		R	K			R	K	K		
48	E	D	D	D		E	D	D	D	D	
53											K
54	Y		Y	Y	Y	Y	Y		Y	Y	
55									Y		
58	D	D	D		D					D	
59						Y				K	
63											D
64											D
65								D			E
66	D		D	D	D	D	D		D		
67	D					E	E				D
70		Y	Y	Y	Y	Y	Y	Y	Y	Y	
72										Y	
76				K		K	K	H	K		
79										D	
80									K		
82	E	E	E	E	E	E	E	E	E		E
84									D		D
86											D
88										R	
90	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	
91	E						E	E	K		Y
93											E
95											E
95d										Y	
95h											H
95i										D	D
97											K
100						K					K
101											R
102	K	K	K	K	K	K	K	K	K	K	
103	K					K	K	K			K
113										Y	
114	D	D	D	D	D	D	D	D	D	D	D
117						D	D				
119	H	H	H	H		H	H	H	H		
121	D	D	D	D	D	D	D	D	D	D	D
133	D	D	D	D		D					K
136				K							
137				R			D		K		

138				E		E					
139							Y				
140a						K					
141											E
143a										E	L
145							D				D
146	R	R	R				R				
147	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
150e										K	
150j										Y	
151							H				K
152									D		
153	R	R	R	R	R	R	K	R	R		
154					D		E	D			
155	E			E		E	E				E
156										Y	
157	D			D	D	D	E			K	
158	R	E									K
159						Y					
160c										R	
160e										D	
160k										R	
161	D					E	E				K
162									K		
164	K	R		K	K	K	K				
165		D				D					E
168	Y	H				H					K
170	R	R	R	R	R	R	R	R	R		R
171		Y	Y								
172	D	D	D	D	D	D	D	D	D	E	
173											E
175	K			K		E			E	E	
176		D	D								D
178	D	D	D	D	D	D	D	D	D	D	
181			D	E		D					K
183									D	K	
184										Y	
185	R	R	R	K		E		R	E	K	K
186		R	R				E			E	R
188		R									K
191						K					K
192	E			E	E		E		E	E	
195			D			D					D
196	R	R	R	R	R		K		R	R	R
200	R		R	K	K	K					
201	R	R	R	R	R	R	R	R	R	R	R

Cellulolytic Activity

The cellulase variants of the invention show improved performance. Some of the variants may show improved performance with respect to increased catalytic activity.

In the context of this invention, cellulase activity can be expressed in S-CEVU. Cellulolytic enzymes hydrolyse CMC, thereby increasing the viscosity of the incubation mixture. The resulting reduction in viscosity may be determined by a vibration viscosimeter (e.g. MIVI 3000 from Sofraser, France).

Determination of the cellulolytic activity, measured in terms of S-CEVU, may be determined according to the following analysis method (assay): The S-CEVU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxy-methylcellulose (CMC). The assay is carried out at 40°C; pH 7.5; 0.1M phosphate buffer; time 30 min; using a relative enzyme standard for reducing the viscosity of the CMC(carboxymethylcellulose Hercules 7 LFD) substrate; enzyme concentration approx. 0.15 S-CEVU/ml. The arch standard is defined to 8200 S-CEVU/g.

Altered Sensitivity to Anionic Tensides

The cellulase variants of the invention show improved performance. Some of the variants may show improved performance with respect to an altered sensitivity towards anionic tensides. Anionic tensides are products frequently incorporated into detergent compositions.

Unfolding of cellulases tested so far, is accompanied by a decay in the intrinsic fluorescence of the proteins. The intrinsic fluorescence derives from Trp side chains (and to a smaller extent Tyr side chains) and is sensitive to the hydrophobicity of the sid chain nvironment. Unfolding l ads to a mor hydrophilic environment as the side-chains become more exposed to solvent, and this qu nches fluorescence.

Fluorescence is followed on a P rkin/Elm r™ LS50 luminescence spectrometer. In practice, the greatest chang in fluorescence on unfolding is obtained by xcitation at 280 nm and emission at 345 nm. Slit widths (which regulate the magnitude of the signal) are usually 5 nm for both emission and excitation at a protein concentration of 5 µg/ml. Fluorescence is measured in 2-ml quartz cuvettes thermostatted with a circulating water bath and stirred with a small magnet. The magnet-stirrer is built into the spectrometer.

Unfolding can be followed in real time using the available software. Rapid unfolding (going to completion within less than 5-10 minutes) is monitored in the TimeDrive option, in which the fluorescence is measured every few (2-5) seconds. For slower unfolding, four cuvettes can be measured at a time in the cuvette-holder using the Wavelength Program option, in which the fluorescence of each cuvette is measured every 30 seconds. In all cases, unfolding is initiated by adding a small volume (typically 50 µl) of concentrated enzyme solution to the thermostatted cuvette solution where mixing is complete within a few seconds due to the rapid rotation of the magnet.

Data are measured in the software program *GraphPad Prism*. Unfolding fits in all cases to a single-exponential function from which a single half-time of unfolding (or unfolding rate constant) can be obtained.

Typical unfolding conditions are:

(A) 10 mM CAPS pH 10, 1000 ppm LAS, 40°C

(B) (B) 10 mM HEPES pH 10, 200 ppm LAS, 25°C.

In both cases, the protein concentration is 5-10 µg/ml (the protein concentration is not crucial, as LAS is in excess). Under these conditions, the unfolding of *Humicola insolens* cellulase can be compared with other enzymes (Table 1). This enables us to draw up the following ranking order for stability against anionic tenside:

Thielavia terrestris/Q119H ≅ *Thielavia terrestris* >> *Humicola insolens* ≅ *Humicola insolens*/H119Q.

Cellulase	$t_{½}$ pH 10 (s) (1000 ppm LAS, 40°C)	$t_{½}$ pH 7 (s) (200 ppm LAS, 25°C)
Humicola insolens	48	28
Humicola insolens/H119Q	63	9[a]

Thielavia terrestris	970	690
Thielavia terrestris/Q119H	1100	550

* Unfolding is double-exponential. The $t_{½}$ of the slower phase is ca. 120 s.

Altered pH Optimum

The cellulase variants of the invention show improved performance. Some of the variants may show improved performance with respect to an altered pH optimum. An altered pH optimum may be determined as described below.

Background: To determine the pH optimum for cellulases we have selected organic buffers because it is common known that e.g. borate forms covalent complexes with mono- and oligo-saccharides and phosphate can precipitate with Ca-ions.
In DATA FOR BIOCHEMICAL RESEARCH Third Edition OXFORD SCIENCE PUBLICATIONS page 223 to 241, suitable organic buffers has been found. In respect of their pK_a values we decided to use Na-acetate in the range 4 - 5.5, MES at 6.0, MOPS in the range 6.5 - 7.5, Na-barbiturate 8.0 - 8.5 and glycine in the range 9.0- 10.5.

Method: The method is enzymatic degradation of carboxy-methyl-cellulose, at different pH's. Buffers are prepared in the range 4.0 to 10.5 with intervals of 0.5 pH unit. The analyze is based on formation of new reducing ends in carboxy-methyl-cellulose, these are visualized by reaction with PHBAH in strong alkaline environment, were they forms a yellow compound with absorption maximum at 410 nm.

Experimental Protocol:

Buffer preparation: 0.2 mol of each buffer substance is weighed out and dissolved in 1 liter of Milli Q water.
250 ml. 0.2M buffer solution and 200 ml. Milli Q water is mixed. The pH are measured using Radiometer PHM92 labmeter calibrated

using standard buffer solutions from Radiometer. The pH of the buffers are adjusted to actual pH using 4M NaOH or 4M HCl and adjusted to total 500 ml with water. When adjusting Na-barbiturate to pH 8.0 there might be some precipitation, this can be re-dissolved by heating to 50°C.

Acetic acid 100% 0.2 mol = 12.01 g.

MES 0.2 mol = 39.04 g.

MOPS 0.2 mol = 41.86 g.

Na-barbiturate 0.2 mol = 41.24 g.

Glycine 0.2mol = 15.01 g.

Buffers:	pH:	4.0, 4.5, 5.0 & 5.5 Na-acetate 0.1M
	pH:	6.0 Na-MES 0.1M
	pH:	6.5, 7.0 & 7.5 Na-MOPS 0.1M
	pH:	8.0 & 8.5 Na-barbiturate 0.1M
	pH:	9.0, 9.5, 10.0 & 10.5 Na.glycine 0.1M

The actual pH is measured in a series treated as the main values, but without stop reagent, pH is measured after 20 min. incubation at 40 °C.

Substrate Preparation: 2.0 g. CMC ,in 250 ml. conic glass flask with a magnet rod, is moistened with 2.5 ml. 96% ethanol, 100 ml. Milli Q water is added and then boiled to transparency on a heating magnetic stirrer. Approximately 2 min. boiling. Cooled to room temperature on magnetic stirrer.

Stop Reagent: 1.5 g. PHBAH and 5 g. K-Na-tartrat dissolved in 2% NaOH

Procedure: There are made 3 main values and 2 blank value using 5 ml glass test tubes. (1 main value for pH determination)

	Main values	Blank value
Buffer	1.0 ml.	1.0 ml.
Substrate CMC	0.75 ml.	0.75 ml.
Mix	5 sec.	5 sec.
Preheat	10 min./40°C.	-
Enzyme	0.25 ml.	-
Mix	5 sec.	-
Incubation	20 min./ 40°C.	room temp.
PHBAH-reagent	1 ml.	1 ml.
Mix	5 sec.	-
Enzyme	-	0.25 ml.
Mix	-	5 sec.

Mixing on a Heidolph REAX 2000 mixer with permanent mix and maximum speed (9). No stirring during incubation on water bath with temperature control. Immediately after adding PHBAH-reagent and mixing the samples are boiled 10 min. Cooled in cold tap water for 5 min. Absorbance read at 410 nm.

Determination of Activity

The absorbance at 410 nm from the 2 Main values are added and divided by 2 and the 2 Blank values are added and divided by 2, the 2 mean values are subtracted. The percentages are calculated by using the highest value as 100%.

The measured pH is plotted against the relative activity.

Reagents:

Name:	Supplier:	Cat. no. :	Batch no. :	pKa	Mw: g/mol
Acetic acid 100%	MERCK	1.00063	K20928263 422	4.76	60.05
MES (2[N-Morpholino]ethanesulfonic Acid)	SIGMA	M-8250	68F-5625	6.09	195.2
MOPS (3-[N-Morpholino]propanesulfonic Acid)	SIGMA	M-1254	115F-5629	7.15	209.3
Na-barbiturate (5,5-Diethylbarbituric acid sodium salt)	MERCK	6318	K20238018 404	7.98	206.2
Glycine	MERCK	4201	K205535601 405	9.78	75.07
PHBAH (p-HYDROXY BENZOIC ACID HYDRAZIDE)	SIGMA	H-9882	53H7704		
K-Na-tartrate (Potassium sodium tartrate tetrahydrate)	MERCK	8087	A653387 304		
NaOH Sodium hydroxyde	MERCK	1.06498	C294798 404		
CMC (Carboxy Methyl Cellulose)	Hercules (FMC)	7LF (nov. 89)			

Enzyme Compositions

In a still further aspect, the present invention relates to an enzyme composition comprising an enzyme exhibiting cellulolytic activity as described above.

The enzyme composition of the invention may, in addition to the cellulase of the invention, comprise one or more other enzyme types, for instance hemi-cellulase such as xylanase and mannanase, other cellulase components, chitinase, lipase, esterase, pectinase, cutinase, phytase, oxidoreductase, protease, or amylase.

The enzyme composition may be prepared in accordance with methods known in the art and may be in the form of a liquid or a dry composition. For instance, the enzyme composition may be in the form of a granulate or a microgranulate. The enzyme to be included in the composition may be stabilized in accordance with methods known in the art.

Examples are given below of preferred uses of th nzym composition of the inv ntion. The dosage of the nzyme composition of the inv ntion and other conditions under which the composition is used may be determined on the basis of methods known in the art.

The enzyme composition according to the invention may be useful for at least one of the following purposes.

Uses

During washing and wearing, dyestuff from dyed fabrics or garment will conventionally bleed from the fabric which then looks faded and worn. Removal of surface fibers from the fabric will partly restore the original colors and looks of the fabric. By the term "color clarification", as used herein, is meant the partly restoration of the initial colors of fabric or garment throughout multiple washing cycles.

The term "de-pilling" denotes removing of pills from the fabric surface.

The term "soaking liquor" denotes an aqueous liquor in which laundry may be immersed prior to being subjected to a conventional washing process. The soaking liquor may contain one or more ingredients conventionally used in a washing or laundering process.

The term "washing liquor" denotes an aqueous liquor in which laundry is subjected to a washing process, i.e. usually a combined chemical and mechanical action either manually or in a washing machine. Conventionally, the washing liquor is an aqueous solution of a powder or liquid detergent composition.

The term "rinsing liquor" denotes an aqueous liquor in which laundry is immersed and treated, conventionally immediately after being subjected to a washing process, in order to rinse the laundry, i.e. essentially remove the detergent solution from the laundry. The rinsing liquor may contain a fabric conditioning or softening composition.

The laundry subjected to the method of the present invention may be conventional washable laundry. Preferably, the major part of the laundry is sewn or un-sewn fabrics, including knits, wovens, denims, yarns, and toweling, made from cotton, cotton blends or natural or manmade cellulosics (.g. originating from xylan-containing cellulose fibers such as from wood pulp) or bl nds ther of. Examples of

blends are bl nds of cotton or rayon/viscose with one or more companion material such as wool, synthetic fibers (e.g. polyamide fibers, acrylic fibers, polyester fibers, polyvinyl alcohol fibers, polyvinyl chloride fibers, polyvinylidene chloride fibers, polyurethane fibers, polyurea fibers, aramid fibers), and cellulose-containing fibers (e.g. rayon/viscose, ramie, flax/linen, jute, cellulose acetate fibers, lyocell).

DETERGENT DISCLOSURE AND EXAMPLES

Surfactant System

The detergent compositions according to the present invention comprise a surfactant system, wherein the surfactant can be selected from nonionic and/or anionic and/or cationic and/or ampholytic and/or zwitterionic and/or semi-polar surfactants.

The surfactant is typically present at a level from 0.1% to 60% by weight.

The surfactant is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated in such a way that it promotes, or at least does not degrade, the stability of any enzyme in these compositions.

Preferred systems to be used according to the present invention comprise as a surfactant one or more of the nonionic and/or anionic surfactants described herein.

Polyethylene, polypropylene, and polybutylene oxide condensates of alkyl phenols are suitable for use as the nonionic surfactant of the surfactant systems of the present invention, with the polyethylene oxide condensates being preferred. These compounds include the condensation products of alkyl phenols having an alkyl group containing from about 6 to about 14 carbon atoms, preferably from about 8 to about 14 carbon atoms, in either a straight chain or branched-chain configuration with the alkylene oxide. In a preferred embodiment, the ethylene oxide is present in an amount qual to from about 2 to about 25 moles, more preferably from about 3 to about 15 moles, of ethylene oxide per mole of alkyl phenol. Commercially available nonionic surfactants of this type include Igepal™ CO-630, mark ted by the GAF Corporation; and Triton™ X-45, X-114, X-100 and X-102, all mark ted by th Rohm & Haas

Company. These surfactants are commonly r ferred to as alkylphenol alkoxylates (.g., alkyl ph nol thoxylates).

The condensation products of primary and secondary aliphatic alcohols with about 1 to about 25 moles of ethylene oxide are suitable for use as the nonionic surfactant of the nonionic surfactant systems of the present invention. The alkyl chain of the aliphatic alcohol can either be straight or branched, primary or secondary, and generally contains from about 8 to about 22 carbon atoms. Preferred are the condensation products of alcohols having an alkyl group containing from about 8 to about 20 carbon atoms, more preferably from about 10 to about 18 carbon atoms, with from about 2 to about 10 moles of ethylene oxide per mole of alcohol. About 2 to about 7 moles of ethylene oxide and most preferably from 2 to 5 moles of ethylene oxide per mole of alcohol are present in said condensation products. Examples of commercially available nonionic surfactants of this type include Tergitol™ 15-S-9 (The condensation product of C_{11}-C_{15} linear alcohol with 9 moles ethylene oxide), Tergitol™ 24-L-6 NMW (the condensation product of C_{12}-C_{14} primary alcohol with 6 moles thylene oxide with a narrow molecular weight distribution), both marketed by Union Carbide Corporation; Neodol™ 45-9 (the condensation product of C_{14}-C_{15} linear alcohol with 9 moles of ethylene oxide), Neodol™ 23-3 (the condensation product of C_{12}-C_{13} linear alcohol with 3.0 moles of ethylene oxide), Neodol™ 45-7 (the condensation product of C_{14}-C_{15} linear alcohol with 7 moles of ethylene oxide), Neodol™ 45-5 (the condensation product of C_{14}-C_{15} linear alcohol with 5 moles of thylene oxide) marketed by Shell Chemical Company, Kyro™ EOB (the condensation product of C_{13}-C_{15} alcohol with 9 moles ethylene oxide), marketed by The Procter & Gamble Company, and Genapol LA 050 (the condensation product of C_{12}-C_{14} alcohol with 5 moles of ethylene oxide) marketed by Hoechst. Preferred range of HLB in thes products is from 8-11 and most preferred from 8-10.

Also useful as the nonionic surfactant of the surfactant systems of the present invention are alkylpolysaccharides disclosed in US 4,565,647, having a hydrophobic group containing from about 6 to about 30 carbon atoms, preferably from about 10 to about 16 carbon atoms and a polysaccharid , .g. a polyglycoside, hydrophilic group containing from about 1.3 to about 10, pref rably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7 saccharide units. Any reducing

saccharide containing 5 or 6 carbon atoms can be used, e.g., glucose, galactose and galactosyl moieties can be substituted for the glucosyl moi ties (optionally the hydrophobic group is attached at the 2-, 3-, 4-, etc. positions thus giving a glucose or galactose as opposed to a glucoside or galactoside). The intersaccharide bonds can be, e.g., between the one position of the additional saccharide units and the 2-, 3-, 4-, and/or 6- positions on the preceding saccharide units.

The preferred alkylpolyglycosides have the formula

$$R^2O(C_nH_{2n}O)_t(\text{glycosyl})_x$$

wherein R^2 is selected from the group consisting of alkyl, alkylphenyl, hydroxyalkyl, hydroxyalkylphenyl, and mixtures thereof in which the alkyl groups contain from about 10 to about 18, preferably from about 12 to about 14, carbon atoms; n is 2 or 3, preferably 2; t is from 0 to about 10, pre-ferably 0; and x is from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7. The glycosyl is preferably derived from glucose. To prepare these compounds, the alcohol or alkylpolyethoxy alcohol is formed first and then reacted with glucose, or a source of glucose, to form the glucoside (attachment at the 1-position). The additional glycosyl units can then be attached between their 1-position and the preceding glycosyl units 2-, 3-, 4-, and/or 6-position, preferably predominantly the 2-position.

The condensation products of ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol are also suitable for use as the additional nonionic surfactant systems of the present invention. The hydrophobic portion of these compounds will preferably have a molecular weight from about 1500 to about 1800 and will exhibit water insolubility. The addition of polyoxyethylene moieties to this hydrophobic portion tends to increase the water solubility of the molecule as a whole, and the liquid character of the product is retained up to the point where the polyoxyethylene content is about 50% of the total weight of the condensation product, which corresponds to condensation with up to about 40 moles of ethylene oxide. Examples of compounds of this type include certain of th comm rcially available Pluronic™ surfactants, marketed by BASF.

Also suitable for use as th nonionic surfactant of the nonionic surfactant system of the present invention, are the condensation products of thylene oxide with

the product r sulting from the reaction of propylene oxid and thyl nediamine. The hydrophobic moiety of these products consists of the reaction product of ethyl-nediamine and excess propylene oxide, and generally has a molecular weight of from about 2500 to about 3000. This hydrophobic moiety is condensed with ethylene oxide to the extent that the condensation product contains from about 40% to about 80% by weight of polyoxyethylene and has a molecular weight of from about 5,000 to about 11,000. Examples of this type of nonionic surfactant include certain of the commercially available Tetronic™ compounds, marketed by BASF.

Preferred for use as the nonionic surfactant of the surfactant systems of the present invention are polyethylene oxide condensates of alkyl phenols, condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethyleneoxide, alkylpolysaccharides, and mixtures hereof. Most preferred are C_8-C_{14} alkyl phenol ethoxylates having from 3 to 15 ethoxy groups and C_8-C_{18} alcohol thoxylates (preferably C_{10} avg.) having from 2 to 10 ethoxy groups, and mixtures thereof.

Highly preferred nonionic surfactants are polyhydroxy fatty acid amide surfactants of the formula

$$\begin{array}{c} R^2 - \underset{\displaystyle \| \atop \displaystyle O}{C} - \underset{\displaystyle \| \atop \displaystyle R^1}{N} - Z, \end{array}$$

wherein R^1 is H, or R^1 is C_{1-4} hydrocarbyl, 2-hydroxyethyl, 2-hydroxypropyl or a mixture thereof, R^2 is C_{5-31} hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R^1 is methyl, R^2 is straight C_{11-15} alkyl or C_{16-18} alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose or lactose, in a reductive amination reaction.

Highly preferred anionic surfactants include alkyl alkoxylated sulfate surfactants. Examples hereof are water soluble salts or acids of the formula $RO(A)_mSO3M$ wherein R is an unsubstitut d C_{10}-C_{24} alkyl or hydroxyalkyl group having a C_{10}-C_{24} alkyl component, preferably a C_{12}-C_{20} alkyl or hydro-xyalkyl, more pref rably C_{12}-C_{18} alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater

than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl, trimethyl-ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and those derived from alkylamines such as ethylamine, diethylamine, triethylamine, mixtures thereof, and the like. Exemplary surfactants are C_{12}-C_{18} alkyl polyethoxylate (1.0) sulfate (C_{12}-$C_{18}E(1.0)M$), C_{12}-C_{18} alkyl polyethoxylate (2.25) sulfate (C_{12}-$C_{18}(2.25)M$, and C_{12}-C_{18} alkyl polyethoxylate (3.0) sulfate (C_{12}-$C_{18}E(3.0)M$), and C_{12}-C_{18} alkyl polyethoxylate (4.0) sulfate (C_{12}-$C_{18}E(4.0)M$), wherein M is conveniently selected from sodium and potassium.

Suitable anionic surfactants to be used are alkyl ester sulfonate surfactants including linear esters of C_8-C_{20} carboxylic acids (i.e., fatty acids) which are sulfonated with gaseous SO_3 according to "The Journal of the American Oil Chemists Society", 52 (1975), pp. 323-329. Suitable starting materials would include natural fatty substances as derived from tallow, palm oil, etc.

The preferred alkyl ester sulfonate surfactant, especially for laundry applications, comprise alkyl ester sulfonate surfactants of the structural formula:

$$R^3 - CH(SO_3M) - C(=O) - OR^4$$

wherein R^3 is a C_8-C_{20} hydrocarbyl, preferably an alkyl, or combination thereof, R^4 is a C_1-C_6 hydrocarbyl, preferably an alkyl, or combination thereof, and M is a cation which forms a water soluble salt with the alkyl ester sulfonate. Suitable salt-forming cations include metals such as sodium, potassium, and lithium, and substituted or unsubstituted ammonium cations, such as monoethanolamine, diethanolamine, and triethanolamine. Preferably, R^3 is C_{10}-C_{16} alkyl, and R^4 is methyl,

ethyl or isopropyl. Especially preferred are the methyl st r sulfonates wherein R^3 is C_{10}-C_{16} alkyl.

Other suitable anionic surfactants include the alkyl sulfate surfactants which are water soluble salts or acids of the formula $ROSO_3M$ wherein R preferably is a C_{10}-C_{24} hydrocarbyl, preferably an alkyl or hydroxyalkyl having a C_{10}-C_{20} alkyl component, more preferably a C_{12}-C_{18} alkyl or hydroxyalkyl, and M is H or a cation, e.g., an alkali metal cation (e.g. sodium, potassium, lithium), or ammonium or substituted ammonium (e.g. methyl-, dimethyl-, and trimethyl ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and quaternary ammonium cations derived from alkylamines such as ethylamine, diethylamine, triethylamine, and mixtures thereof, and the like). Typically, alkyl chains of C_{12}-C_{16} are preferred for lower wash temperatures (e.g. below about 50°C) and C_{16}-C_{18} alkyl chains are preferred for higher wash temperatures (e.g. above about 50°C).

Other anionic surfactants useful for detersive purposes can also be included in the laundry detergent compositions of the present invention. Theses can include salts (including, for example, sodium, potassium, ammonium, and substituted ammonium salts such as mono- di- and triethanolamine salts) of soap, C_8-C_{22} primary or secondary alkanesulfonates, C_8-C_{24} olefinsulfonates, sulfonated polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkaline earth metal citrates, e.g., as described in British patent specification No. 1,082,179, C_8-C_{24} alkylpolyglycolethersulfates (containing up to 10 moles of ethylene oxide); alkyl glycerol sulfonates, fatty acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol thylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates such as the acyl isethionates, N-acyl taurates, alkyl succinamates and sulfosuccinates, monoesters of sulfosuccinates (especially saturated and unsaturated C_{12}-C_{18} monoesters) and diesters of sulfosuccinates (especially saturated and unsaturated C_6-C_{12} diesters), acyl sarcosinates, sulfates of alkylpolysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, and alkyl polyethoxy carboxylates such as those of the formula $RO(CH_2CH_2O)_k$-CH_2C00-M+ wher in R is a C_8-C_{22} alkyl, k is an integer from 1 to 10, and M is a soluble salt forming cation. Resin acids and hydrogenated

resin acids are also suitable, such as rosin, hydrogenated rosin, and r sin acids and hydrogenated resin acids present in or derived from tall oil.

Alkylbenzene sulfonates are highly preferred. Especially preferred are linear (straight-chain) alkyl benzene sulfonates (LAS) wherein the alkyl group preferably contains from 10 to 18 carbon atoms.

Further examples are described in "Surface Active Agents and Detergents" (Vol. I and II by Schwartz, Perrry and Berch). A variety of such surfactants are also generally disclosed in US 3,929,678, (Column 23, line 58 through Column 29, line 23, herein incorporated by reference).

When included therein, the laundry detergent compositions of the present invention typically comprise from about 1% to about 40%, preferably from about 3% to about 20% by weight of such anionic surfactants.

The laundry detergent compositions of the present invention may also contain cationic, ampholytic, zwitterionic, and semi-polar surfactants, as well as the nonionic and/or anionic surfactants other than those already described herein.

Cationic detersive surfactants suitable for use in the laundry detergent compositions of the present invention are those having one long-chain hydrocarbyl group. Examples of such cationic surfactants include the ammonium surfactants such as alkyltrimethylammonium halogenides, and those surfactants having the formula:

$$[R^2(OR^3)_y][R^4(OR^3)_y]_2R^5N+X-$$

wherein R^2 is an alkyl or alkyl benzyl group having from about 8 to about 18 carbon atoms in the alkyl chain, each R^3 is selected form the group consisting of $-CH_2CH_2-$, $-CH_2CH(CH_3)-$, $-CH_2CH(CH_2OH)-$, $-CH_2CH_2CH_2-$, and mixtures thereof; each R^4 is selected from the group consisting of C_1-C_4 alkyl, C_1-C_4 hydroxyalkyl, benzyl ring structures formed by joining the two R^4 groups, $-CH_2CHOHCHOHCOR^6CHOHCH_2OH$, wherein R^6 is any hexose or hexose polymer having a molecular weight less than about 1000, and hydrogen when y is not 0; R^5 is the same as R^4 or is an alkyl chain,wherein the total number of carbon atoms or R^2 plus R^5 is not more than about 18; each y is from 0 to about 10,and the sum of the y values is from 0 to about 15; and X is any compatibl anion.

Highly pr f rred cationic surfactants are the water solubl quaternary ammonium compounds useful in the present composition having th formula:

$R_1R_2R_3R_4N^+X^-$ (i)

wher in R_1 is C_8-C_{16} alkyl, ach of R_2, R_3 and R_4 is independently C_1-C_4 alkyl, C_1-C_4 hydroxy alkyl, benzyl, and $-(C_2H_{40})_xH$ where x has a value from 2 to 5, and X is an anion. Not more than one of R_2, R_3 or R_4 should be benzyl.

The preferred alkyl chain length for R_1 is C_{12}-C_{15}, particularly where the alkyl group is a mixture of chain lengths derived from coconut or palm kernel fat or is derived synthetically by olefin build up or OXO alcohols synthesis.

Preferred groups for R_2R_3 and R_4 are methyl and hydroxyethyl groups and the anion X may be selected from halide, methosulphate, acetate and phosphate ions.

Examples of suitable quaternary ammonium compounds of formulae (i) for use herein are:

coconut trimethyl ammonium chloride or bromide;

coconut methyl dihydroxyethyl ammonium chloride or bromide;

decyl triethyl ammonium chloride;

decyl dimethyl hydroxyethyl ammonium chloride or bromide;

C_{12-15} dimethyl hydroxyethyl ammonium chloride or bromide;

coconut dimethyl hydroxyethyl ammonium chloride or bromide;

myristyl trimethyl ammonium methyl sulphate;

lauryl dimethyl benzyl ammonium chloride or bromide;

lauryl dimethyl $(ethenoxy)_4$ ammonium chloride or bromide;

choline esters (compounds of formula (i) wherein R_1 is

CH_2-CH_2-O-C-C_{12-14} alkyl and $R_2R_3R_4$ are methyl).

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di-alkyl imidazolines [compounds of formula (i)].

Other cationic surfactants useful herein are also described in US 4,228,044 and in EP 000 224.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 25%, preferably from about 1% to about 8% by weight of such cationic surfactants.

Ampholytic surfactants are also suitabl for use in the laundry detergent compositions of the present invention. Th se surfactants can be broadly described as

aliphatic derivatives of secondary or tertiary amines, or aliphatic derivatives of heterocyclic secondary and tertiary amines in which the aliphatic radical can be straight- or branched-chain. One of the aliphatic substituents contains at least about 8 carbon atoms, typically from about 8 to about 18 carbon atoms, and at least one contains an anionic water-solubilizing group, e.g. carboxy, sulfonate, sulfate. See US 3,929,678 (column 19, lines 18-35) for examples of ampholytic surfactants.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such ampholytic surfactants.

Zwitterionic surfactants are also suitable for use in laundry detergent compositions. These surfactants can be broadly described as derivatives of secondary and tertiary amines, derivatives of heterocyclic secondary and tertiary amines, or derivatives of quaternary ammonium, quaternary phosphonium or tertiary sulfonium compounds. See US 3,929,678 (column 19, line 38 through column 22, line 48) for examples of zwitterionic surfactants.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such zwitterionic surfactants.

Semi-polar nonionic surfactants are a special category of nonionic surfactants which include water-soluble amine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; watersoluble phosphine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms.

Semi-polar nonionic detergent surfactants include the amine oxide surfactants having the formula:

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↑

$R^3(OR^4)xN(R^5)2$

wher in R^3 is an alkyl, hydroxyalkyl, or alkyl ph nyl group or mixtures thereof containing from about 8 to about 22 carbon atoms; R^4 is an alkylene or hydroxyalkylene group containing from about 2 to about 3 carbon atoms or mixtures thereof; x is from 0 to about 3: and each R^5 is an alkyl or hydroxyalkyl group containing from about 1 to about 3 carbon atoms or a polyethylene oxide group containing from about 1 to about 3 ethylene oxide groups. The R^5 groups can be attached to each other, e.g., through an oxygen or nitrogen atom, to form a ring structure.

These amine oxide surfactants in particular include C_{10}-C_{18} alkyl dimethyl amine oxides and C_8-C_{12} alkoxy ethyl dihydroxy ethyl amine oxides.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such semi-polar nonionic surfactants.

Builder System

The compositions according to the present invention may further comprise a builder system. Any conventional builder system is suitable for use herein including aluminosilicate materials, silicates, polycarboxylates and fatty acids, materials such as ethylenediamine tetraacetate, metal ion sequestrants such as aminopolyphosphonates, particularly ethylenediamine tetramethylene phosphonic acid and diethylene triamine pentamethylenephosphonic acid. Though less preferred for obvious environmental reasons, phosphate builders can also be used herein.

Suitable builders can be an inorganic ion exchange material, commonly an inorganic hydrated aluminosilicate material, more particularly a hydrated synthetic zeolite such as hydrated zeolite A, X, B, HS or MAP.

Another suitable inorganic builder material is layered silicate, e.g. SKS-6 (Hoechst). SKS-6 is a crystalline layered silicate consisting of sodium silicate ($Na_2Si_2O_5$).

Suitable polycarboxylates containing one carboxy group include lactic acid, glycolic acid and ther derivatives thereof as disclosed in Belgian Pat nt Nos. 831,368, 821,369 and 821,370. Polycarboxylat s containing two carboxy groups include

th water-soluble salts of succinic acid, malonic acid, (thylenedioxy) diacetic acid, maleic acid, diglycollic acid, tartaric acid, tartronic acid and fumaric acid, as well as the ether carboxylates described in German Offenle-enschrift 2,446,686, and 2,446,487, US 3,935,257 and the sulfinyl carboxylates described in Belgian Patent No. 840,623. Polycarboxylates containing three carboxy groups include, in particular, water-soluble citrates, aconitrates and citraconates as well as succinate derivatives such as the carboxymethyloxysuccinates described in British Patent No. 1,379,241, lactoxysuccinates described in Netherlands Application 7205873, and the oxypolycarboxylate materials such as 2-oxa-1,1,3-propane tricarboxylates described in British Patent No. 1,387,447.

Polycarboxylates containing four carboxy groups include oxydisuccinates disclosed in British Patent No. 1,261,829, 1,1,2,2,-ethane tetracarboxylates, 1,1,3,3-propane tetracarboxylates containing sulfo substituents include the sulfosuccinate derivatives disclosed in British Patent Nos. 1,398,421 and 1,398,422 and in US 3,936,448, and the sulfonated pyrolysed citrates described in British Patent No. 1,082,179, while polycarboxylates containing phosphone substituents are disclosed in British Patent No. 1,439,000.

Alicyclic and heterocyclic polycarboxylates include cyclopentane-cis,cis-cis-tetracarboxylates, cyclopentadienide pentacarboxylates, 2,3,4,5-tetrahydro-furan - cis, cis, cis-tetracarboxylates, 2,5-tetrahydro-furan-cis, discarboxylates, 2,2,5,5,-tetrahydrofuran - tetracarboxylates, 1,2,3,4,5,6-hexane - hexacarboxylates and carboxymethyl derivatives of polyhydric alcohols such as sorbitol, mannitol and xylitol. Aromatic polycarboxylates include mellitic acid, pyromellitic acid and the phthalic acid derivatives disclosed in British Patent No. 1,425,343.

Of the above, the preferred polycarboxylates are hydroxy-carboxylates containing up to three carboxy groups per molecule, more particularly citrates.

Preferred builder systems for use in the present compositions include a mixture of a water-insoluble aluminosilicate builder such as zeolite A or of a layered silicate (SKS-6), and a water-soluble carboxylate chelating agent such as citric acid.

A suitabl chelant for inclusion in th detergent composi-ions in accordance with the inv ntion is thylenediamine-N,N'-disuccinic acid (EDDS) or the alkali metal, alkalin arth metal, ammonium, or substituted ammonium salts th reof, or mixtures

thereof. Preferred EDDS compounds are the free acid form and the sodium or magnesium salt thereof. Examples of such preferred sodium salts of EDDS include Na_2EDDS and Na_4EDDS. Examples of such preferred magnesium salts of EDDS include MgEDDS and Mg_2EDDS. The magnesium salts are the most preferred for inclusion in compositions in accordance with the invention.

Preferred builder systems include a mixture of a water-insoluble aluminosilicate builder such as zeolite A, and a water soluble carboxylate chelating agent such as citric acid.

Other builder materials that can form part of the builder system for use in granular compositions include inorganic materials such as alkali metal carbonates, bicarbonates, silicates, and organic materials such as the organic phosphonates, amino polyalkylene phosphonates and amino polycarboxylates.

Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated form each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

Detergency builder salts are normally included in amounts of from 5% to 80% by weight of the composition. Preferred levels of builder for liquid detergents are from 5% to 30%.

Enzymes

Preferred detergent compositions, in addition to the enzyme preparation of the invention, comprise other enzyme(s) which provides cleaning performance and/or fabric care benefits.

Such enzymes include proteases, lipases, cutinases, amylases, cellulases, peroxidases, oxidases (e.g. laccases)

Proteases: Any protease suitable for use in alkaline solutions can be used. Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically or genetically modified mutants are included. The

prot ase may be a serine protease, pr f rably an alkalin microbial protease or a trypsin-like protease. Examples of alkaline prot ases are subtilisins, specially those derived from Bacillus, e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the Fusarium protease described in WO 89/06270.

Preferred commercially available protease enzymes include those sold under the trade names Alcalase, Savinase, Primase, Durazym, and Esperase by Novo Nordisk A/S (Denmark), those sold under the tradename Maxatase, Maxacal, Maxapem, Properase, Purafect and Purafect OXP by Genencor International, and those sold under the tradename Opticlean and Optimase by Solvay Enzymes. Protease enzymes may be incorporated into the compositions in accordance with the invention at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of enzyme protein by weight of the composition.

Lipases: Any lipase suitable for use in alkaline solutions can be used. Suitable lipases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included.

Examples of useful lipases include a Humicola lanuginosa lipase, e.g., as described in EP 258 068 and EP 305 216, a Rhizomucor miehei lipase, e.g., as described in EP 238 023, a Candida lipase, such as a C. antarctica lipase, e.g., the C. antarctica lipase A or B described in EP 214 761, a Pseudomonas lipase such as a P. alcaligenes and P. pseudoalcaligenes lipase, e.g., as described in EP 218 272, a P. cepacia lipase, e.g., as described in EP 331 376, a P. stutzeri lipase, e.g., as disclosed in GB 1,372,034, a P. fluorescens lipase, a Bacillus lipase, e.g., a B. subtilis lipase (Dartois et al., (1993), Biochemica et Biophysica acta 1131, 253-260), a B. stearothermophilus lipase (JP 64/744992) and a B. pumilus lipase (WO 91/16422).

Furthermor , a number of clon d lipases may be useful, including the P nicillium camembertii lipase described by Yamaguchi et al., (1991), Gene 103, 61-67), the Geotricum candidum lipas (Schimada, Y. t al., (1989), J. Bioch m., 106,

383-388), and various Rhizopus lipases such as a R. d lemar lipase (Hass, M.J et al., (1991), Gene 109, 117-113), a R. niv us lipase (Kugimiya t al., (1992), Biosci. Biotech. Biochem. 56, 716-719) and a R. oryzae lipase.

Other types of lipolytic enzymes such as cutinases may also be useful, e.g., a cutinase derived from Pseudomonas mendocina as described in WO 88/09367, or a cutinase derived from Fusarium solani pisi (e.g. described in WO 90/09446).

Especially suitable lipases are lipases such as M1 Lipase™, Luma fast™ and Lipomax™ (Genencor), Lipolase™ and Lipolase Ultra™ (Novo Nordisk A/S), and Lipase P "Amano" (Amano Pharmaceutical Co. Ltd.).

The lipases are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of nzyme protein by weight of the composition.

Amylases: Any amylase (a and/or b) suitable for use in alkaline solutions can be used. Suitable amylases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. Amylases include, for example, a-amylases obtained from a special strain of B. licheniformis, described in more detail in GB 1,296,839. Commercially available amylases are Duramyl™, Termamyl™, Fungamyl™ and BAN™ (available from Novo Nordisk A/S) and Rapidase™ and Maxamyl P™ (available from Genencor).

The amylases are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of nzyme protein by weight of the composition.

Cellulases: Any cellulase suitable for use in alkaline solutions can be used. Suitable cellulases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included. Suitabl cellulas s ar disclosed in US 4,435,307, which discloses fungal cellulases produced from Humicola insolens.

Especially suitable cellulases are the cellulases having colour car benefits. Examples of such cellulases are cellulas s described in European patent application No. 0 495 257 and the endoglucanase of the present invention.

Commercially available cellulases include Celluzyme™ produced by a strain of Humicola insolens, (Novo Nordisk A/S), and KAC-500(B)™ (Kao Corporation).

Cellulases are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of enzyme protein by weight of the composition.

Peroxidases/Oxidases :Peroxidase enzymes are used in combination with hydrogen peroxide or a source thereof (e.g. a percarbonate, perborate or persulfate). Oxidase enzymes are used in combination with oxygen. Both types of enzymes are used for "solution bleaching", i.e. to prevent transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, preferably together with an enhancing agent as described in e.g. WO 94/12621 and WO 95/01426. Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically or genetically modified mutants are included.

Peroxidase and/or oxidase enzymes are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of enzyme protein by weight of the composition.

Mixtures of the above mentioned enzymes are encompassed herein, in particular a mixture of a protease, an amylase, a lipase and/or a cellulase.

The enzyme of the invention, or any other enzyme incorporated in the detergent composition, is normally incorporated in the deterg nt composition at a level from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a l v l from 0.0001% to 1% of enzyme protein by weight of th composition, more

preferably at a l vel from 0.001% to 0.5% of enzym protein by weight of the composition, v n mor preferably at a lev l from 0.01% to 0.2% of nzyme protein by weight of the composition.

Bleaching Agents

Additional optional detergent ingredients that can be included in the detergent compositions of the present invention include bleaching agents such as PB1, PB4 and percarbonate with a particle size of 400-800 microns. These bleaching agent components can include one or more oxygen bleaching agents and, depending upon the bleaching agent chosen, one or more bleach activators. When present oxygen bleaching compounds will typically be present at levels of from about 1% to about 25%. In general, bleaching compounds are optional added components in non-liquid formulations, e.g. granular detergents.

The bleaching agent component for use herein can be any of the bleaching agents useful for detergent compositions including oxygen bleaches as well as others known in the art.

The bleaching agent suitable for the present invention can be an activated or non-activated bleaching agent.

One category of oxygen bleaching agent that can be used encompasses percarboxylic acid bleaching agents and salts thereof. Suitable examples of this class of agents include magnesium monoperoxyphthalate hexahydrate, the magnesium salt of meta-chloro perbenzoic acid, 4-nonylamino-4-oxoperoxybutyric acid and diperoxydodecanedioic acid. Such bleaching agents are disclosed in US 4,483,781, US 740,446, EP 0 133 354 and US 4,412,934. Highly preferred bleaching agents also include 6-nonylamino-6-oxoperoxycaproic acid as described in US 4,634,551.

Another category of bleaching agents that can be used encompasses the halogen bleaching agents. Examples of hypohalite bleaching agents, for example, include trichloro isocyanuric acid and the sodium and potassium dichloroisocyanurates and N-chloro and N-bromo alkane sulphonamides. Such materials are normally added at 0.5-10% by w ight of the finished product, preferably 1-5% by weight.

The hydrogen peroxide r leasing agents can be used in combination with bleach activators such as tetra-acetyl thyl n diamine (TAED), nonanoyloxybenzene-sulfonate (NOBS, described in US 4,412,934), 3,5-trimethyl-hexsanoloxybenzenesulfonate (ISONOBS, described in EP 120 591) or pentaacetylglucose (PAG), which are perhydrolyzed to form a peracid as the active bleaching species, leading to improved bleaching effect. In addition, very suitable are the bleach activators C8(6-octanamido-caproyl) oxybenzene-sulfonate, C9(6-nonanamido caproyl) oxybenzenesulfonate and C10 (6-decanamido caproyl) oxybenzenesulfonate or mixtures thereof. Also suitable activators are acylated citrate sters such as disclosed in European Patent Application No. 91870207.7.

Useful bleaching agents, including peroxyacids and bleaching systems comprising bleach activators and peroxygen bleaching compounds for use in cleaning compositions according to the invention are described in application USSN 08/136,626.

The hydrogen peroxide may also be present by adding an enzymatic system (i.e. an enzyme and a substrate therefore) which is capable of generation of hydrogen peroxide at the beginning or during the washing and/or rinsing process. Such enzymatic systems are disclosed in European Patent Application EP 0 537 381.

Bleaching agents other than oxygen bleaching agents are also known in the art and can be utilized herein. One type of non-oxygen bleaching agent of particular interest includes photoactivated bleaching agents such as the sulfonated zinc and/or aluminium phthalocyanines. These materials can be deposited upon the substrate during the washing process. Upon irradiation with light, in the presence of oxygen, such as by hanging clothes out to dry in the daylight, the sulfonated zinc phthalocyanine is activated and, consequently, the substrate is bleached. Preferred zinc phthalocyanine and a photoactivated bleaching process are described in US 4,033,718. Typically, detergent composition will contain about 0.025% to about 1.25%, by weight, of sulfonated zinc phthalocyanine.

Bleaching agents may also comprise a manganese catalyst. Th mangan s catalyst may, .g., be on of the compounds described in "Efficient mangan se catalysts for low-temperatur bleaching", Nature 369, 1994, pp. 637-639.

Sud Suppressors

Another optional ingredient is a suds suppressor, x mplified by silicones, and silica-silicone mixtures. Silicones can generally be represented by alkylated polysiloxane materials, while silica is normally used in finely divided forms exemplified by silica aerogels and xerogels and hydrophobic silicas of various types. Theses materials can be incorporated as particulates, in which the suds suppressor is advantageously releasably incorporated in a water-soluble or waterdispersible, substantially non surface-active detergent impermeable carrier. Alternatively the suds suppressor can be dissolved or dispersed in a liquid carrier and applied by spraying on to one or more of the other components.

A preferred silicone suds controlling agent is disclosed in US 3,933,672. Other particularly useful suds suppressors are the self-emulsifying silicone suds suppressors, described in German Patent Application DTOS 2,646,126. An example of such a compound is DC-544, commercially available form Dow Corning, which is a siloxane-glycol copolymer. Especially preferred suds controlling agent are the suds suppressor system comprising a mixture of silicone oils and 2-alkyl-alkanols. Suitable 2-alkyl-alkanols are 2-butyl-octanol which are commercially available under the trade name Isofol 12 R.

Such suds suppressor system are described in European Patent Application EP 0 593 841.

Especially preferred silicone suds controlling agents are described in European Patent Application No. 92201649.8. Said compositions can comprise a silicone/ silica mixture in combination with fumed nonporous silica such as Aerosil^{R}.

The suds suppressors described above are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight.

Other Components

Other components used in detergent compositions may be employed such as soil-suspending ag nts, soil-releasing ag nts, optical brighteners, abrasives, bactericides, tarnish inhibitors, coloring agents, and/or ncapsulated or nonencapsulated perfum s.

Especially suitable ncapsulating materials are wat r soluble capsules which consist of a matrix of polysaccharide and polyhydroxy compounds such as described in GB 1,464,616.

Other suitable water soluble encapsulating materials comprise dextrins derived from ungelatinized starch acid esters of substituted dicarboxylic acids such as described in US 3,455,838. These acid-ester dextrins are, preferably, prepared from such starches as waxy maize, waxy sorghum, sago, tapioca and potato. Suitable xamples of said encapsulation materials include N-Lok manufactured by National Starch. The N-Lok encapsulating material consists of a modified maize starch and glucose. The starch is modified by adding monofunctional substituted groups such as octenyl succinic acid anhydride.

Antiredeposition and soil suspension agents suitable herein include cellulose derivatives such as methylcellulose, carboxymethylcellulose and hydroxyethylcellulose, and homo- or co-polymeric polycarboxylic acids or their salts. Polymers of this type include the polyacrylates and maleic anhydride-acrylic acid copolymers previously mentioned as builders, as well as copolymers of maleic anhydride with ethylene, methylvinyl ether or methacrylic acid, the maleic anhydride constituting at least 20 mole percent of the copolymer. These materials are normally used at levels of from 0.5% to 10% by weight, more preferably form 0.75% to 8%, most preferably from 1% to 6% by weight of the composition.

Preferred optical brighteners are anionic in character, examples of which are disodium 4,4'-bis-(2-diethanolamino-4-anilino -s- triazin-6-ylamino)stilbene-2:2' disulphonate, disodium 4, - 4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino-stilbene-2:2' - disulphonate, disodium 4,4' - bis-(2,4-dianilino-s-triazin-6-ylamino)stilbene-2:2' - disulphonate, monosodium 4',4'' - bis-(2,4-dianilino-s-tri-azin-6 ylamino)stilbene-2-sulphonate, disodium 4,4' -bis-(2-anilino-4-(N-methyl-N-2-hydroxyethylamino)-s-triazin-6-ylamino)stilbene-2,2' - disulphonate, di-sodium 4,4' -bis-(4-phenyl-2,1,3-triazol-2-yl)-stilbene-2,2' disulphonate, di-so-dium 4,4'bis(2-anilino-4-(1-methyl-2-hydroxyethylamino)-s-triazin-6-ylami-no)stilbene-2,2'disulphonate, sodium 2(stilbyl-4''-(naphtho-1',2':4,5)-1,2,3, - triazole-2''-sulphonate and 4,4'-bis(2-sulphostyryl)biphenyl.

Oth r useful polymeric materials are the polyethylen glycols, particularly those of molecular weight 1000-10000, more particularly 2000 to 8000 and most

preferably about 4000. Th se are used at levels of from 0.20% to 5% more preferably from 0.25% to 2.5% by weight. These polymers and th previously m ntioned homo- or co-polymeric poly-carboxylate salts ar valuable for improving whit n ss maintenance, fabric ash deposition, and cleaning performance on clay, proteinaceous and oxidizable soils in the presence of transition metal impurities.

Soil release agents useful in compositions of the present invention are conventionally copolymers or terpolymers of terephthalic acid with ethylene glycol and/or propylene glycol units in various arrangements. Examples of such polymers are disclosed in US 4,116,885 and 4,711,730 and EP 0 272 033. A particular preferred polymer in accordance with EP 0 272 033 has the formula:

$$(CH_3(PEG)_{43})_{0.75}(POH)_{0.25}[T\text{-}PO)_{2.8}(T\text{-}PEG)_{0.4}]T(POH)_{0.25}((PEG)_{43}CH_3)_{0.75}$$

where PEG is $-(OC_2H_4)0-$, PO is (OC_3H_6O) and T is $(pOOC_6H_4CO)$.

Also very useful are modified polyesters as random copolymers of dimethyl terephthalate, dimethyl sulfoisophthalate, ethylene glycol and 1,2-propanediol, the end groups consisting primarily of sulphobenzoate and secondarily of mono esters of thylene glycol and/or 1,2-propanediol. The target is to obtain a polymer capped at both end by sulphobenzoate groups, "primarily", in the present context most of said copolymers herein will be endcapped by sulphobenzoate groups. However, some copolymers will be less than fully capped, and therefore their end groups may consist of monoester of ethylene glycol and/or 1,2-propanediol, thereof consist "secondarily" of such species.

The selected polyesters herein contain about 46% by weight of dimethyl terephthalic acid, about 16% by weight of 1,2-propanediol, about 10% by weight thylene glycol, about 13% by weight of dimethyl sulfobenzoic acid and about 15% by weight of sulfoisophthalic acid, and have a molecular weight of about 3.000. The polyesters and their method of preparation are described in detail in EP 311 342.

Softening Agents

Fabric softening agents can also be incorporated into laundry detergent compositions in accordance with the pr sent invention. These agents may be inorganic or organic in type. Inorganic softening agents are xemplified by the smectite clays disclosed in GB-A-1 400898 and in US 5,019,292. Organic fabric

softening agents include the water insoluble tertiary amines as disclosed in GB-A1 514 276 and EP 0 011 340 and th ir combination with mono C_{12}-C_{14} quaternary ammonium salts are disclosed in EP-B-0 026 528 and di-long-chain amides as disclosed in EP 0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP 0 299 575 and 0 313 146.

Levels of smectite clay are normally in the range from 5% to 15%, more preferably from 8% to 12% by weight, with the material being added as a dry mixed component to the remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or dilong chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight. These materials are normally added to the spray dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as molten liquid on to other solid components of the composition.

Polymeric Dye-transfer Inhibiting Agents

The detergent compositions according to the present invention may also comprise from 0.001% to 10%, preferably from 0.01% to 2%, more preferably form 0.05% to 1% by weight of polymeric dye- transfer inhibiting agents. Said polymeric dye-transfer inhibiting agents are normally incorporated into detergent compositions in order to inhibit the transfer of dyes from colored fabrics onto fabrics washed therewith. These polymers have the ability of complexing or adsorbing the fugitive dyes washed out of dyed fabrics before the dyes have the opportunity to become attached to other articles in the wash.

Especially suitable polymeric dye-transfer inhibiting agents are polyamine N-oxide polymers, copolymers of N-vinyl-pyrrolidone and N-vinylimidazole, polyvinyl-pyrrolidone polymers, polyvinyloxazolidones and polyvinylimidazol s or mixtures thereof.

Addition of such polymers also enhances the performance of the enzymes according the invention.

The detergent composition according to the invention can be in liquid, paste, gels, bars or granular forms.

Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591.

Granular compositions according to the present invention can also be in "compact form", i.e. they may have a relatively higher density than conventional granular detergents, i.e. form 550 to 950 g/l; in such case, the granular detergent compositions according to the present invention will contain a lower amount of "Inorganic filler salt", compared to conventional granular detergents; typical filler salts are alkaline earth metal salts of sulphates and chlorides, typically sodium sulphate; "Compact" detergent typically comprise not more than 10% filler salt. The liquid compositions according to the present invention can also be in "concentrated form", in such case, the liquid detergent compositions according to the present invention will contain a lower amount of water, compared to conventional liquid detergents. Typically, the water content of the concentrated liquid detergent is less than 30%, more preferably less than 20%, most preferably less than 10% by weight of the detergent compositions.

The compositions of the invention may for example, be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the pretreatment of stained fabrics, rinse added fabric softener compositions, and compositions for use in general household hard surface cleaning operations and dishwashing operations.

The following examples are meant to exemplify compositions for the present invention, but are not necessarily meant to limit or otherwise define the scope of the invention.

In the detergent compositions, the abbreviated component identifications have the following meanings:

LAS: Sodium linear C_{12} alkyl benzene sulphonate

TAS: Sodium tallow alkyl sulphate

XYAS: Sodium C_{1X} - C_{1Y} alkyl sulfate

SS: Secondary soap surfactant of formula 2-butyl octanoic acid

25EY: A C_{12} - C_{15} predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide

45EY: A C_{14} - C_{15} predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide

XYEZS: C_{1X} - C_{1Y} sodium alkyl sulfate condensed with an average of Z moles of ethylene oxide per mole

Nonionic: C_{13} - C_{15} mixed ethoxylated/propoxylated fatty alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5 sold under the tradename Plurafax LF404 by BASF Gmbh

CFAA: C_{12} - C_{14} alkyl N-methyl glucamide

TFAA: C_{16} - C_{18} alkyl N-methyl glucamide

Silicate: Amorphous Sodium Silicate (SiO_2:Na_2O ratio = 2.0)

NaSKS-6: Crystalline layered silicate of formula d-$Na_2Si_2O_5$

Carbonate: Anhydrous sodium carbonate

Phosphate: Sodium tripolyphosphate

MA/AA: Copolymer of 1:4 maleic/acrylic acid, average molecular weight about 80,000

Polyacrylate: Polyacrylate homopolymer with an average molecular weight of 8,000 sold under the tradename PA30 by BASF Gmbh

Zeolite A: Hydrated Sodium Aluminosilicate of formula $Na_{12}(AlO_2SiO_2)_{12}$. $27H_2O$ having a primary particle size in the range from 1 to 10 micrometers

Citrat : Tri-sodium citrat dihydrate

Citric: Citric Acid

P rborate: Anhydrous sodium perborate monohydrate bleach, empirical formula $NaBO_2.H_2O_2$

PB4: Anhydrous sodium perborate tetrahydrate

Percarbonate: Anhydrous sodium percarbonate bleach of empirical formula $2Na_2CO_3.3H_2O_2$

TAED: Tetraacetyl ethylene diamine

CMC: Sodium carboxymethyl cellulose

DETPMP: Diethylene triamine penta (methylene phosphonic acid), marketed by Monsanto under the Tradename Dequest 2060

PVP: Polyvinylpyrrolidone polymer

EDDS: Ethylenediamine-N, N'-disuccinic acid, [S,S] isomer in the form of the sodium salt

Suds Suppressor: 25% paraffin wax Mpt 50°C, 17% hydrophobic silica, 58% paraffin oil

Granular Suds suppressor: 12% Silicone/silica, 18% stearyl alcohol, 70% starch in granular form

Sulphate: Anhydrous sodium sulphate

HMWPEO: High molecular weight polyethylene oxide

TAE 25: Tallow alcohol ethoxylate (25)

Detergent Example I

A granular fabric cleaning composition in accordance with the invention may be prepared as follows:

Sodium linear C_{12} alkyl benzene sulfonate	6.5
Sodium sulfate	15.0
Zeolite A	26.0
Sodium nitrilotriacetate	5.0
Enzyme of the invention	0.1
PVP	0.5
TAED	3.0
Boric acid	4.0

P rborat	18.0
Phenol sulphonat	0.1
Minors	Up to 100

Detergent Example II

A compact granular fabric cleaning composition (density 800 g/l) in accord with the invention may be prepared as follows:

45AS	8.0
25E3S	2.0
25E5	3.0
25E3	3.0
TFAA	2.5
Zeolite A	17.0
NaSKS-6	12.0
Citric acid	3.0
Carbonate	7.0
MA/AA	5.0
CMC	0.4
Enzyme of the invention	0.1
TAED	6.0
Percarbonate	22.0
EDDS	0.3
Granular suds suppressor	3.5
water/minors	Up to 100%

Detergent Example III

Granular fabric cleaning compositions in accordance with the invention which are especially useful in the laundering of coloured fabrics were prepared as follows:

LAS	10.7	-
TAS	2.4	-
TFAA	-	4.0
45AS	3.1	10.0

45E7	4.0	-
25E3S	-	3.0
68E11	1.8	-
25E5	-	8.0
Citrate	15.0	7.0
Carbonate	-	10
Citric acid	2.5	3.0
Zeolite A	32.1	25.0
Na-SKS-6	-	9.0
MA/AA	5.0	5.0
DETPMP	0.2	0.8
Enzyme of the invention	0.10	0.05
Silicate	2.5	-
Sulphate	5.2	3.0
PVP	0.5	-
Poly (4-vinylpyridine)-N-Oxide/copolymer of vinyl-imidazole and vinyl-pyrrolidone	-	0.2
Perborate	1.0	-
Phenol sulfonate	0.2	-
Water/Minors	Up to 100%	

Detergent Example IV

Granular fabric cleaning compositions in accordance with the invention which provide "Softening through the wash" capability may be prepared as follows:

45AS	-	10.0
LAS	7.6	-
68AS	1.3	-
45E7	4.0	-
25E3	-	5.0
Coco-alkyl-dim thyl hydroxy-	1.4	1.0

thyl ammonium chloride		
Citrate	5.0	3.0
Na-SKS-6	-	11.0
Zeolite A	15.0	15.0
MA/AA	4.0	4.0
DETPMP	0.4	0.4
Perborate	15.0	-
Percarbonate	-	15.0
TAED	5.0	5.0
Smectite clay	10.0	10.0
HMWPEO	-	0.1
Enzyme of the invention	0.10	0.05
Silicate	3.0	5.0
Carbonate	10.0	10.0
Granular suds suppressor	1.0	4.0
CMC	0.2	0.1
Water/Minors	Up to 100%	

Detergent Example V

Heavy duty liquid fabric cleaning compositions in accordance with the invention may be prepared as follows:

	I	II
LAS acid form	-	25.0
Citric acid	5.0	2.0
25AS acid form	8.0	-
25AE2S acid form	3.0	-
25AE7	8.0	-
CFAA	5	-
DETPMP	1.0	1.0
Fatty acid	8	-
Oleic acid	-	1.0
Ethanol	4.0	6.0

Propanediol	2.0	6.0
Enzyme of the inv ntion	0.10 0.05	
Coco-alkyl dimethyl hydroxy ethyl ammonium chloride	-	3.0
Smectite clay	-	5.0
PVP	2.0	-
Water / Minors	Up to 100%	

Textile Applications

In another embodiment, the present invention relates to use of the endoglucanase of the invention in the bio-polishing process. Bio-Polishing is a specific treatment of the yarn surface which improves fabric quality with respect to handl and appearance without loss of fabric wettability. The most important effects of Bio-Polishing can be characterized by less fuzz and pilling, increased gloss/luster, improved fabric handle, increased durable softness and altered water absorbency. Bio-Polishing usually takes place in the wet processing of the manufacture of knitted and woven fabrics. Wet processing comprises such steps as e.g. desizing, scouring, bleaching, washing, dying/printing and finishing. During each of these steps, the fabric is more or less subjected to mechanical action. In general, after the textiles have been knitted or woven, the fabric proceeds to a desizing stage, followed by a scouring stage, etc. Desizing is the act of removing size from textiles. Prior to weaving on mechanical looms, warp yarns are often coated with size starch or starch derivatives in order to increase their tensile strength. After weaving, the size coating must be removed before further processing the fabric in order to ensure a homogeneous and wash-proof result. It is known that in order to achieve the effects of Bio-Polishing, a combination of cellulytic and mechanical action is required. It is also known that "super-softness" is achievable when the treatment with a cellulase is combined with a conventional treatment with softening agents. It is contemplated that use of the ndoglucanase of the invention for bio-polishing of cellulosic fabrics is advantageous, .g. a more thorough polishing can be achieved. Bio-polishing may be obtained by applying the m thod described e.g. in WO 93/20278.

Ston -Wa hing

It is known to provide a "stone-washed" look (localized abrasion of th colour) in dyed fabric, especially in denim fabric or jeans, either by washing the denim or jeans made from such fabric in the presence of pumice stones to provide the desired localized lightening of the colour of the fabric or by treating the fabric enzymatically, in particular with cellulytic enzymes. The treatment with an endoglucanase of the present invention may be carried out either alone such as disclosed in US 4,832,864, together with a smaller amount of pumice than required in the traditional process, or together with perlite such as disclosed in WO 95/09225.

Pulp and Paper Applications

In the papermaking pulp industry, the endoglucanase of the present invention may be applied advantageously e.g. as follows:

- For debarking: pretreatment with the endoglucanase may degrade the cambium layer prior to debarking in mechanical drums resulting in advantageous nergy savings.

- For defibration: treatment of a material containing cellulosic fibers with the ndoglucanase prior to refining or beating may result in reduction of the energy consumption due to the hydrolysing effect of the cellulase on the interfibre surfaces. Use of the endoglucanase may result in improved energy savings as compared to the use of known enzymes, since it is believed that the enzyme composition of the invention may possess a higher ability to penetrate fibre walls.

- For fibre modification, i.e. improvement of fibre properties where partial hydrolysis across the fibre wall is needed which requires deeper penetrating enzymes (e.g. in order to make coarse fibers more flexible). Deep treatment of fibers has so far not been possible for high yield pulps e.g. mechanical pulps or mixtures of recycled pulps. This has been ascribed to the nature of the fibre wall structure that prevents the passage of enzyme molecules due to physical restriction of the pore matrix of the fibre wall. It is contemplated that the present ndoglucanase is capable of pen trating into th fibre wall.

- For drainage improvement. The drainability of papermaking pulps may be improved by treatment of the pulp with hydrolysing enzym s, .g. cellulases. Use of the present endoglucanase may be more ffective, e.g. result in a high r degree of loosening bundles of strongly hydrated micro-fibrils in the fines fraction (consisting of fibre debris) that limits the rate of drainage by blocking hollow spaces between fibers and in the wire mesh of the paper machine. The Canadian standard freeness (CSF) increases and the Schopper-Riegler drainage index decreases when pulp in subjected to cellulase treatment, see e.g. US patent 4,923,565; TAPPI T227, SCAN C19:65.- nce.

- For inter fibre bonding. Hydrolytic enzymes are applied in the manufacture of papermaking pulps for improving the inter fibre bonding. The enzymes rinse the fibre surfaces for impurities e.g. cellulosic debris, thus enhancing the area of exposed cellulose with attachment to the fibre wall, thus improving the fibre-to-fibre hydrogen binding capacity. This process is also referred to as dehornification. Paper and board produced with a cellulase containing enzyme preparation may have an improved strength or a reduced grammage, a smoother surface and an improved printability.

- For enzymatic deinking. Partial hydrolysis of recycled paper during or upon pulping by use of hydrolysing enzymes such as cellulases are known to facilitate the removal and agglomeration of ink particles. Use of the present endoglucanse may give a more effective loosening of ink from the surface structure due to a better pen- tration of the enzyme molecules into the fibrillar matrix of the fibre wall, thus softening the surface whereby ink particles are effectively loosened. The agglomeration of loosened ink particles are also improved, due to a more efficient hydrolysis of cellulosic fragments found attached to ink particles originating from the fibres.

The treatment of lignocellulosic pulp may, e.g., be performed as described in WO 91/14819, WO 91/14822, WO 92/17573 and WO 92/18688.

Degradation of Plant Material

In y t anoth r mbodiment, the present invention relates to use of the ndoglucanase and/or nzyme preparation according to the inv ntion for degradation of plant material e.g. cell walls.

It is contemplated that the novel endoglucanase and/or enzyme preparation of the invention is useful in the preparation of wine, fruit or vegetable juice in order to increase yield. Endoglucanases according to the invention may also be applied for enzymatic hydrolysis of various plant cell-wall derived materials or waste materials, e.g. agricultural residues such as wheat-straw, corn cobs, whole corn plants, nut shells, grass, vegetable hulls, bean hulls, spent grains, sugar beet pulp, and the like. The plant material may be degraded in order to improve different kinds of processing, facilitate purification or extraction of other components like purification of beta-glucan or beta-glucan oligomers from cereals, improve the feed value, decrease the water binding capacity, improve the degradability in waste water plants, improve the conversion of e.g. grass and corn to ensilage, etc.

EXAMPLES

The invention is further illustrated in the following examples which are not intended to be in any way limiting to the scope of the invention as claimed.

EXAMPLE 1

Multiple Sequence Alignment of Cellulases

The multiple sequence alignment is performed using the *Pileup* algorithm as implemented in the Wisconsin Sequence Analysis Package version 8.1-UNIX (GCG, Genetics Computer Group, Inc.). The method used is similar to the method described by *Higgens and Sharp* (CARBIOS 1989 **5** 151-153). A gap creation penalty of 3.0 and a gap extension penalty of 0.1 is used together with a scoring matrix as described in Nucl. Acids Res. 1986 **14** (16) 6745-6763 (Dayhoff table *(Schwartz, R. M. and Dayhoff, M. O.;* Atlas of Protein Sequence and Structure (Dayhoff, M. O. Ed.); National Biomedical Research Foundation, Washington D.C. 1979 353-358) rescaled by dividing each value by the sum of its row and column, and normalizing to a mean of 0 and standard deviation of 1.0. The value for FY (Phe-Tyr) = RW = 1.425. Perfect matches are set to 1.5 and no matches on any row are better than perfect matches).

Pair-wise Sequence Alignment of Cellulases

A pair-wise sequence alignment is performed using the algorithm described by *Needleman & Wunsch* (J. Mol. Biol.1970 **48** 443-453), as implemented in the GAP routine in the Wisconsin Sequence Analysis Package (GCG). The parameters used for the GAP routine are the same as mentioned for the Pileup routine earlier.

Pair-wise Sequence Alignment of Cellulases with Forced Pairing

A pair-wise sequence alignment with forced pairing of residues is performed using the algorithm described by *Needleman & Wunsch* (J. Mol. Biol. 1970 **48** 443-453), as implemented in the GAP routine in the Wisconsin Sequence Analysis Package (GCG). The parameters used for the GAP routine are the same as mentioned for the Pileup routine earlier, where the scoring matrix is modified to incorporate a residue named X which symbolize the residues to be paired. The diagonal value for X paired with X is set to 9.0 and all off diagonal values involving X is set to 0.

Complex between *Humicola insolens* Endoglucanase and Celloheptaose

Based on the X-ray structure of the core domain of the *Humicola insolens* EGV family 45 endoglucanase inactive variant (D10N) in complex with cellohexaose (*Davies et.al.*; Biochemistry 1995 **34**16210-12220, PDB entry 4ENG) a model of the structure of the native *Humicola insolens* EGV family 45 endoglucanase core domain in complex with celloheptaose is build using the following steps:

1. Using the Biopolymer module of the Insight II 95.0 (Insight II 95.0 User Guide, October 1995. San Diego: Biosym/MSI, 1995) replace N10 with a aspartic acid.
2. Make a copy of the sugar unit occupying subsite -3 by copying all the molecule and delete the extra atoms. Manually move the new sugar unit to best fit the unoccupied -1 binding site. Create the bonds to bind the new sugar unit to the two existing cellotriose units.
3. Delete overlapping crystal water molecules. These are identified by using the Subset Interface By_Atom 2.5 command.
4. Build hydrogens at a pH of 8.0 and applying charged terminals.

5. Protonate D121 using the Residue Replace <D121 residue name> ASP L command.
6. Apply the CVFF forcefield template through the command Potentials Fix.
7. Fix all atoms except the new sugar unit.
8. Relax the atomic position of the new sugar unit using 300 cycles of simple energy minimization followed by 5000 steps of 1fs simple molecular dynamics ending by 300 cycles of simple energy minimization all using the molecular mechanics program Discover 95.0/3.0.1 (Discover 95.0/3.0.0 User Guide, October 1995. San Diego: Biosym/MSI, 1995.).

Homology Building of Cellulases

The construction of a structural model of a family 45 cellulase with known amino acid sequence based on a known X-ray structure of the *Humicola insolens* EGV family 45 cellulase consists of the following steps:

1. Define the approximate extend of the core region of the structure to be modeled and the alignment of the cysteine based on multiple sequence alignment between many known family 45 cellulase sequences.
2. Pair-wise sequence alignment between the new sequence and the sequence of the known X-ray structure.
3. Define Structurally Conserved Regions (SCRs) based on the sequence alignment.
4. Assign coordinates for the model structure within the SCRs.
5. Find structures for the loops or Variable Regions (VRs) between the SCRs by a search in a loop structure database.
6. Assign coordinates for the VRs in the model structure from the database search result.
7. Create disulfide bonds and set protonation state.
8. Refine the build structure using molecular mechanics.

The known X-ray structure of the *Humicola insolens* EGV family 45 cellulase will in the following be termed the reference structure. The structure to be modeled will be termed the model structure.

Ad 1: The approximate extent of the core part of the enzyme is determined by a multiple sequence alignment including many known family 45 cellulase sequences. Since the reference structure only contain atomic coordinat s for the core part of the enzyme only the residues in the sequence to be modeled which align with the core part of the reference structure can be included in the model building. This alignment also determines the alignment of the cysteine. The multiple sequence alignment is performed using the Pileup algorithm as described earlier.

Ad 2: A pair-wise sequence alignment is performed as described earlier. If the cysteine in the conserved disulfide bridges and/or the active site residues (D10 and D121) does not align, a pair-wise sequence alignment using forced pairing of the cysteines in the conserved disulfide bridges and/or the active site residues is performed as described arlier. The main purpose of the sequence alignment is to define SCRs (see later) to be used for a model structure generation.

Ad 3: Based on the sequence alignment Structurally Conserved Regions (SCRs) are defined as continuous regions of overlapping sequence with no insertions or deletions.

Ad 4: Using the computer program Homology 95.0 (Homology User Guide, October 1995. San Diego: Biosym/MSI, 1995.) atomic coordinates in the model structure can be generated from the atomic coordinates of the reference structure using the command AssignCoords Sequences.

Ad 5: Using the computer program Homology 95.0 possible conformations for the remaining regions, named Variable Regions (VRs) are found by a search in the loop structure database included in Homology 95.0. This procedure is performed for each VR.

Ad 6: If the VR length is smaller than six residues the first loop structure in the database search result is selected for coordinate generation. In cases where longer loops are generated the first solution in the list which does not have severe atomic overlap are selected. The degree of atomic overlap can be analyzed using the Bump Monitor Add Intra command in the computer program Insight II 95.0 (Insight II 95.0 User Guide, October 1995. San Diego: Biosym/MSI, 1995.) a parameter of 0.25 for the Bump command will show the severe overlap. If more than ten bumps exists between the inserted loop region and the remaining part of the protein the next solution is tested. If no solution is found with these parameters, the solution with the fewest bumps is selected.

The coordinates for the VR regions are generated using the command AssignCoords Loops in the program Homology 95.0.

Ad 7: The disulfide bonds are created using the Bond Create command in the Biopolymer module of Insight II 95.0 and the protonation state is set to match pH 8.0 with charged caps using the Hydrogens command. Finally the active proton donor (the residue quivalent to D121 in the reference structure) is protonated using the residue replace <D121 residue name> ASP L command. To finalize the data of the model the appropriate forcefield template is applied using the CVFF forcefield through the command Potentials Fix.

Ad 8: Finally the modeled structure is subjected to 500 cycles energy minimization using the molecular mechanics program Discover 95.0/3.0.1 (Discover 95.0/3.0.0 User Guide, October 1995. San Diego: Biosym/MSI, 1995.). The output from the above described procedure is atomic coordinates describing a structural model for the core domain of a new family 45 cellulase based on sequence homology to the *Humicola insolens* EGV family 45 cellulase.

Superpositioning of 45 Cellulase Structures

To overlay two family 45 cellulase structures a superposition of the structures are performed using the Structure Alignment command of the Homology 95.0 (Homology User Guide, October 1995. San Diego: Biosym/MSI, 1995.). All parameters for the command are chosen as the default values.

Determination of Residues within 3Å and 5Å from the Substrate

In order to determine the amino acid residues within a specified distance from the substrate, a given family 45 cellulase structure is superimposed on the cellulase part of the model structure of the complex between *Humicola insolens* EGV family 45 endoglucanase and celloheptaose as described above. The residues within a specified distance of the substrate are then found using the Interface Subset command of the Insight II 95.0 (Insight II 95.0 User Guide, October 1995. San Diego: Biosym/MSI, 1995). The specified distance are supplied as parameter to the program.

The results of this determination are presented in Tables 2 and 3, abov .

Determination of Surface Accessibility

To determine the solvent accessibility the Access_Surf command in Homology 95.0 (Homology User Guide, October 1995; San Diego: Biosym/MSI, 1995) was used. The program uses the definition proposed by *Lee and Richards* (*Lee, B. & Richards, F.M.* "The interpretation of protein structures: Estimation of static accessibility", J.Mol.Biol. 1971 **55** 379-400). A solvent probe radius of 1.4 Å was used and only heavy atoms (i.e. non-hydrogen atoms) were included in the calculation. Residues with zero accessibility is defined as being buried, all other residues are defined as being solvent exposed and on the surface of the enzyme structure.

Transferring Level of Specific Activity between Cellulases

In order to transfer the level of catalytic activity between two family 45 cellulases, the following protocol is applied using the methods described above. This method will pinpoint amino acid residues responsible for the difference in specific activity, and one or more of those amino acid residues must be replaced in one sequence in order to transfer the level of specific activity from the comparison family 45 cellulase:

1) Perform multiple sequence alignment of all known family 45 cellulases (excluding *Trichoderma reesei*). From this identify conserved disulfide bridges amongst the two involved sequences and the sequence of the *Humicola insolens* EGV cellulase are identified and the active site residues (D10 and D121) are located;
2) Perform pair-wise sequence alignment of each sequence with the *Humicola insolens* EGV cellulase core domain (residues 1-201). If the cysteines in the conserved disulfide bridges does not align at the same positions and/or if the two active site residues (D10 and D121) does not align at the same positions then use the pair-wise sequence alignment of Family 45 cellulases with forced pairing method. Include only residues in the sequences overlapping with the core domain (residues 1-201) of the *Humicola insolens* EGV cellulase;
3) Cr ate a homology build structure of each sequence;

4) Determination of residues within 3Å from the substrat in each of th homology build structures. Differences between the sequences in these positions will most probably be the residues responsibl for the difference in specific activity. In the case where residues in inserts are found in any of the sequences within the above mentioned distance, the complete insert can be responsible for the difference in specific activity, and the complete insert must be transferred to the sequence without the insert or the complete insert must be deleted in the sequence with the insert;

5) If not all specific activity was restored by substitution of residues within 3Å of the substrate, determination of residues within 5Å from the substrate in each of the homology build structures will reveal the most probable residues responsible for the remaining difference in specific activity. In the case where residues in inserts are found in any of the sequences within the above mentioned distance, the complete insert can be responsible for the difference in specific activity, and the complete insert must be transferred to the sequence without the insert or the complete insert must be deleted in the sequence with the insert.

Transferring the Level of Stability towards Anionic Tensides between Cellulases

In order to transfer level of stability towards anionic tensides between two family 45 cellulases, the following protocol is applied using the methods described above. This method will pinpoint amino acid residues responsible for the difference in level of stability towards anionic tensides, and one or more of those amino acid residues must be replaced in one sequence in order to transfer the level of specific activity from the comparison family 45 cellulase:

1) Perform multiple sequence alignment of all known family 45 cellulases (excluding *Trichoderma reesei*). From this identify conserved disulfide bridges amongst the two involved sequences and the sequence of the *Humicola insolens* EGV cellulase are identified and the active site residues (D10 and D121) are located;

2) Perform pair-wise sequence alignment of each sequ nce with the *Humicola insolens* EGV cellulase core domain (residu s 1-201). If the

cysteines in the conserved disulfide bridges does not align at th same positions and/or if the two active site r sidues (D10 and D121) does not align at the same positions then use the pair-wise sequence alignment of Family 45 cellulases with forced pairing method. Include only residues in the sequences overlapping with the core domain (residues 1-201)of the *Humicola insolens* EGV cellulase;

3) Create a homology build structure of each sequence;

4) Determination of residues located at the surface of the enzyme. This is done by calculation the surface accessibility. Residues with a surface accessibility greater than 0.0Å^2 are exposed to the surface;

5) Any residue exposed to the surface belonging to the following group of amino acids: D, E, H, K, R and C if not involved in disulfide bridge which differ between the two sequences will most probably be responsible for the difference in level of stability towards anionic tensides. In the case where residues in inserts are found in any of the sequences within the above mentioned group of amino acid types, the complete insert can be responsible for the difference in level of stability towards anionic tensides, and the complete insert must be transferred to the sequence without the insert or the complete insert must be deleted in the sequence with the insert.

EXAMPLE 2

Preparation of Cellulase Variants

Based on the sequence alignment and the computer modeling experiment described in Example 1, position 119 was identified as a particular point of interest for making cellulase variants. Position 119 (cellulase numbering) is located within 3 Å from the substrate. In position 119 the wild-type *Humicola insolens* cellulase holds a histidine residue (H), whereas the wild-type *Thielavia terrestris* cellulase holds a glutamine residue (Q).

In this experiment, histidine was substituted for glutamine in the *Thielavia terrestris* cellulase (thereby obtaining the cellulase variant *Thielavia terrestris*/Q119H). The variant obtained was tested for specific activity.

All *Humicola insolens* variants are, unless otherwise stated, constructed by application of the Chameleon™ Double-stranded, site-directed Mutagenesis kit, from Stratagene. The following synthetic oligo-nucleotide were used as selection primer :

S/M GAATGACTTGGTTGACGCGTCACCAGTCAC, or

M/S GAATGACTTGGTTGAGTACTCACCAGTCAC.

S/M replaces the ScaI site in the beta-lactamase gene of the plasmid with a MluI site and M/S does the reverse. The later is used to introduce secondary mutations in variants generated by the first selection primer.

For construction of *Thielavia terrestis* cellulase variants, the *Thielavia terrestis* EG V cellulase cDNA obtainable from the plasmid deposited as DSM 100811 was used. DSM 100811 was deposited on 30. June 1995 according to the Budapest Treaty. The plasmid was digested with the restriction endonucleases BamHI and NotI The 4153 bp vector part and the1211 bp BamHI-NotI fragment were isolated. Equal portions of the 1211 bp fragment were digested with respectively HgiAI and EcoRV and the 487 bp BamHI - HgiAI and 690 bp EcoRV- NotI fragments were isolated.

These fragments and the vector part were ligated in the presence of 5 fold molar excess of a synthetic DNA fragment, resulting from the annealing of two single stranded DNA oligomers:

18802: CACTGGCGGCGACCTGGGATCTAACCACTTCGAT

18803: ATCGAAGTGGTTAGATCCCAGGTCGCCGCCTGTGCTC

The ligation mixture was transformed into *E. coli* strain XL1, and from the resulting transformants *Thielavia terrestris*/Q119H was isolated and verified by DNA sequencing.

All the cellulase variants ware produced by cloning the gene and transforming the gene into *Aspergillus oryzae* using a plasmid with the gene inserted between the fungal amylase promoter and the AMG terminator from *A. niger*. [Christensen, T. Wöldike, H. Boel, E., Mortensen, S. B., Hjortshøj, K., Thim, L. and Hansen, M.T. (1988) Biotechnology 6: 1419-1422].

The cellulases with a cellulose binding domain CBD were purified by exploiting th ir binding to Avicel. The cloned product was recov red after fermentation by separation of the extracellular fluid from the production organism. Th cellulase was then highly purified by affinity chromatography using 150 gram of Avicel in a

slurry with 20 mM sodiumphosphat pH 7.5. Th Avicel slurry was mix d with the crude f rm ntation broth which in total contains about 1 gram of protein. After mixing at 4° C for 20 min, the Avicel-bound enzyme is packed into a column with a dimension of 50 times 200 mm about 400 ml total.

The column is washed with the 200 ml buffer, then washed with 0.5 M NaCl in the same buffer until no more protein elutes, and washed with 500 ml buffer (20 mM Tris pH 8.5). Finally the pure full length enzyme is eluted with 1% Triethylamine pH 11.8. The eluted enzyme solution is adjusted to pH 8 and concentrated using a Amicon cell unit with a membrane DOW GR61PP (polypropylene with a cut off of 20 KD) to 5 mg protein per ml. The enzymes have all been purified yielding a single band on SDS-PAGE.

Cellulases which natural lack CBD or the linker has been proteolytic cleaved or in which the CBD has been removed by introducing a stop codon after the catalytic domain, can not be purified using Avicel. The extracellular proteins are recovered free from the production organism. The core cellulases were purified free of *Aspergillus* proteins by cation exchange chromatography. The fermentation broth was adjusted to pH 3.5 and filtered to remove the precipitating proteins. Then the proteins were ultra filtrated (concentrated and washed with water) on a DOW GR81PP membrane with a cut off 6 KD until the conductivity of the eluate is below 1000 mS/cm. The sample was finally applied to a S-Sepharose column equilibrated with a 20 mM citrate buffer pH 3.5 .

The enzyme will bind to the S-Sepharose at this low pH and it is eluted as a single peak using a NaCl gradient from 0 to 500 mM. The eluted pure enzyme was concentrated on a Amicon cell with the DOW GR81PP membrane.

All purified cellulases gave a single band in SDS-PAGE.

The specific activity data are summarized in the following table:

Enzyme/variant	Specific activity [ECU/mg]
Humicola isolens	430
Thielavia terrestris	150
Thielavia terrestris/Q119H	394

From this experiment it is seen that by introducing the mutation Q119H into the *Thielavia terrestris* cellulase, the specific activity if the resulting cellulase variants was increased to the level of that of the homologous *Humicola insolens* cellulase.

5

CLAIMS

1. A cellulase variant derived from a parental cellulase by substitution, insertion and/or deletion, which variant has a catalytic core domain, in which the variant
   - at position 5 (cellulase numbering) holds an alanine residue (A), a serine residue (S), or a threonine residue (T);
   - at position 6 (cellulase numbering) holds a threonine residue (T);
   - at position 7 (cellulase numbering) holds an arginine residue (R);
   - at position 8 (cellulase numbering) holds a phenylalanine residue (F), or a tyrosine residue (Y);
   - at position 9 (cellulase numbering) holds a phenylalanine residue (F), a tryptophan residue (W), or a tyrosine residue (Y);
   - at position 10 (cellulase numbering) holds an aspartic acid residue (D);
   - at position 119 (cellulase numbering) holds a holds a histidine residue (H); and
   - at position 121 (cellulase numbering) holds an aspartic acid residue (D).

2. A cellulase variant, which variant holds 4 or more of the following disulfide bridges: C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering).

3. The cellulase variant according to claim 2, which variant holds 5 or more of the following disulfide bridges: C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering).

4. The cellulase variant according to claim 2, which variant holds 6 or more of the following disulfide bridges: C11-C135; C12-C47; C16-C86; C31-C56; C87-C199; C89-C189; and C156-C167 (cellulase numbering).

5. Th cellulas variant according to any of claims 2-4, in which variant a cysteine residue has been replaced by a different natural amino acid residue at on or more of the positions 16, 86, 87, 89, 189, and/or 199 (cellulase numbering).

6. A cellulase variant, derived from a parental cellulase by substitution, insertion and/or deletion at one or more amino acid residues located in the substrate binding cleft at a position within an enzyme-substrate interactive distance from the substrate.

7. The cellulase variant according to claim 6, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more amino acid residues located in the substrate binding cleft at a distance of not more than 5 Å from the substrate.

8. The cellulase variant according to claim 7, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 21a, 42, 44, 45, 47, 48, 49, 49a, 49b, 74, 82, 95j, 110, 111, 112, 113, 114, 115, 116, 119, 121, 123, 127, 128, 129, 130, 131, 132, 132a, 133, 145, 146, 147, 148, 149, 150b, 178, and/or 179 (cellulase numbering).

9. The cellulase variant according to claim 7, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 4, 5, 13, 14, 15, 16, 19, 20, 21, 21a, 42, 44, 47, 48, 49, 49a, 49b, 74, 82, 95j, 110, 111, 113, 115, 116, 119, 123, 129, 131, 132a, 133, 145, 146, 150b, 178, and/or179 (cellulase numbering).

10. The cellulase variant according to claim 6, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more amino acid residues located in the substrate binding cleft at a distance of not more than 3 Å from the substrate.

11. The cellulase variant according to claim 10, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 6, 7, 8, 10, 12, 13, 14, 15, 18, 20, 21, 45, 48, 74, 110, 111, 112,

113, 114, 115, 119, 121, 127, 128, 129, 130, 131, 132, 132a, 146, 147, 148, 150b, 178, and/or 179 (cellulase numbering).

12. The cellulase variant according to claim 10, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 13, 14, 15, 20, 21, 48, 74, 110, 111, 113, 115, 119, 129, 131, 146, 150b, 178, and/or 179 (cellulase numbering).

13. A cellulase variant, in which variant an amino acid residue has been changed into a conserved amino acid residue at one or more positions according to Table 1, at which position(s) between 7 and 10 amino acid residues of the 11 residues identified in Table 1, are identical.

14. The cellulase variant according to claim 13, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 13, 14, 15, 20, 21, 22, 24, 28, 32, 34, 45, 48, 50, 53, 54, 62, 63, 64, 65, 66, 68, 69, 70, 71, 72, 73, 74, 75, 79, 85, 88, 90, 92, 93, 95, 96, 97, 98, 99, 104, 106, 110, 111, 113, 115, 116, 118, 119, 131, 134, 138, 140, 146, 152, 153, 163, 166, 169, 170, 171, 172, 173, 174, 174, 177, 178,179, 180, 193, 196, and/or 197 (cellulase numbering).

15. A cellulase variant, which variant has been derived from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions (cellulase numbering), and which variant:

in position 4 holds R, H, K, Q, V, Y, or M;
in position 5 holds S, T, or A;
in position 13 holds K, or L;
in position 14 holds P, or A;
in position 15 holds H, or S;
in position 16 holds C, or A;
in position 19 holds A, D, S, P, T, or E;
in position 20 holds A, E, G, or K;

in position 21holds K, or N;
in position 21a holds V or *;
in position 22 holds A, G, or P;
in position 24 holds L, V, or *;
in position 28 holds A, L, or V;
in position 32 holds D, K, N, or S;
in position 34 holds D or N;
in position 38 holds F, I, L, or Q;
in position 42 holds D, G, T, N, S, K, or *;
in position 44 holds K, V, R, Q, G, or P ;
in position 45 holds N, or S;
in position 46 holds G, or S;
in position 47 holds C, or Q;
in position 48 holds D, E, N, or S;
in position 49 holds P, S, A, G, or *;
in position 49a holds C, or *;
in position 49b holds N, or *;
in position 50 holds G, or N;
in position 53 holds A, G, K, or S;
in position 54 holds F, or Y;
in position 62 holds F, or W;
in position 63 holds A, or D;
in position 64 holds D, I, or V;
in position 65 holds D, E, N, or S;
in position 68 holds D, N, P, or T;
in position 69 holds A, S, or T;
in position 70 holds L, or Y;
in position 71 holds A, or G;
in position 72 holds F, W, or Y;
in position 73 holds A, or G;
in position 74 holds A, or F;
in position 75 holds A, G, T, or V;

in position 79 holds G, or T;
in position 82 holds E, or *;
in position 88 holds A, G, Q, or R;
in position 90 holds F, or Y;
in position 92 holds A, or L;
in position 93 holds E, Q, or T;
in position 95 holds E, or T;
in position 95j holds P, or *;
in position 96 holds S, or T;
in position 97 holds A, G, or T;
in position 98 holds A, or P;
in position 99 holds L, or V;
in position 104 holds L, or M;
in position 106 holds F, or V;
in position 110 holds N, or S;
in position 111 holds I, T, or V;
in position 113 holds G, or Y;
in position 115 holds L, or V;
in position 116 holds G, Q, or S;
in position 118 holds G, N, Q, or T;
in position 119 holds H, N, or Q;
in position 129 holds L, or V;
in position 131 holds A, I, or L;
in position 132 holds A, P, or T;
in position 133 holds D, K, N, or Q;
in position 134 holds A, or G;
in position 138 holds E, or Q;
in position 145 holds A, D, N, or Q;
in position 146 holds Q, or R;
in position 150b holds A, or *;
in position 152 holds D, or S;
in position 153 holds A, K, L, or R;

in position 163 holds L, V, or W;
in position 166 holds G, or S;
in position 169 holds F, or W;
in position 170 holds F, or R;
in position 171 holds A, F, or Y;
in position 172 holds D, E, or S;
in position 173 holds E, or W;
in position 174 holds F, M, or W;
in position 177 holds A, or N;
in position 178 holds D, or P;
in position 179 holds N, or V;
in position 180 holds L, or P;
in position 193 holds I, or L;
in position 196 holds I, K, or R; and/or
in position 197 holds S, or T.

16. A cellulase variant, which variant comprises one or more of the following mutations (cellulase numbering):

K13L or L13K;
P14A or A14P;
S15H or H15S;
K20E, K20G, K20A, E20K, G20K, A20K, E20G, E20A, G20E, A20E, G20A, or A20G;
K21N or N21K;
A22G, A22P, G22A, P22A, G22P, or P22G;
V24*, V24L, *24V, L24V, *24L, or L24*;
V28A, V28L, A28V, L28V, A28L, or L28A;
N32D, N32S, N32K, D32N, S32N, K32N, D32S, D32K, S32D, K32D, S32K, or K32S;
N34D or D34N;
I38L, I38F, I38Q, L38I, F38I, Q38I, L38F, L38Q, F38L, Q38L, F38Q, or Q38F;

S45N or N45S;

G46S or S46G;

E48D, E48N, D48E, N48E, D48N, or N48D;

G50N or N50G;

A53S, A53G, A53K, S53A, G53A, K53A, S53G, S53K, G53S, K53S, G53K, or K53G;

Y54F or F54Y;

W62F or F62W;

A63D or D63A;

V64I, V64D, I64V, D64V, I64D, or D64I;

N65S, N65D, N65E, S65N, D65N, E65N, S65D, S65E, D65S, E65S, D65E, or E65D;

D66N, D66P, D66T, N66D, P66D, T66D, N66P, N66T, P66N, T66N, P66T, or T66P;

F68V, F68L, F68T, F68P, V68F, L68F, T68F, P68F, V68L, V68T, V68P, L68V, T68V, P68V, L68T, L68P, T68L, P68L, T68P, or P68T;

A69S, A69T, S69A, T69A, S69T, or T69S;

L70Y or Y70L;

G71A or A71G;

F72W, F72Y, W72F, Y72F, W72Y, or Y72W;

A73G or G73A;

A74F or F74A;

T75V, T75A, T75G, V75T, A75T, G75T, V75A, V75G, A75V, G75V, A75G, or G75A;

G79T or T79G;

W85T or T85W;

A88Q, A88G, A88R, Q88A, G88A, R88A, Q88G, Q88R, G88Q, R88Q, G88R, or R88G;

Y90For F90Y;

L92A or A92L;

T93Q, T93E, Q93T, E93T, Q93E, or E93Q;

T95E or E95T;

S96T or T96S;

G97T, G97A, T97G, A97G, T97A, or A97T;

P98A or A98P;

V99L or L99V;

M104L or L104M;

V106F or F106V;

S110N or N110S;

T111I, T111V, I111T, V111T, I111V, or V111I;

G113Y or Y113G;

L115V or V115L;

G116S, G116Q, S116G, Q116G, S116Q, or Q116S;

N118T, N118G, N118Q, T118N, G118N, Q118N, T118G, T118Q, G118T, Q118T, G118Q, or Q118G;

H119Q, H119N, Q119H, or N119H;

V129L or L129V;

I131L, I131A, L131I, A131I, L131A, or A131L;

G134A or A134G;

Q138E or E138Q;

G140N or N140G;

R146Q or Q146R;

S152D or D152S;

R153K, R153L, R153A, K153R, L153R, A153R, K153L, K153A, L153K, A153K, L153A, or A153L;

L163V, L163W, V163L, W163L, V163W, or W163V;

G166S or S166G;

W169F or F169W;

R170F or F170R;

F171Y, F171A, Y171F, A171F, Y171A, or A171Y;

D172E, D172S, E172D, S172D, E172S, or S172E;

W173E or E173W;

F174M, F174W, M174F, W174F, M174W, or W174M;

A177N or N177A;

D178P or P178D;
N179V or V179N;
P180L or L180P;
L193I or I193L;
R196I, R196K, I196R, K196R, I196K, or K196I; and/or
T197S or S197T.

17. A cellulase variant having an altered anion tenside sensitivity, and which variant is from a parental cellulase by substitution, insertion and/or deletion at one or more of the following positions: 2, 4, 7, 8, 10, 13, 15, 19, 20, 21, 25, 26, 29, 32, 33, 34, 35, 37, 40, 42, 42a, 43, 44, 48, 53, 54, 55, 58, 59, 63, 64, 65, 66, 67, 70, 72, 76, 79, 80, 82, 84, 86, 88, 90, 91, 93, 95, 95d, 95h, 95j, 97, 100, 101, 102, 103, 113, 114, 117, 119, 121, 133, 136, 137, 138, 139, 140a, 141, 143a, 145, 146, 147, 150e, 150j, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160c, 160e, 160k, 161, 162, 164, 165, 168, 170, 171, 172, 173, 175, 176, 178, 181, 183, 184, 185, 186, 188, 191, 192, 195, 196, 200, and/or 201 (cellulase numbering).

18. A cellulase variant, in which variant an amino acid residue has been substituted at one or more of the following positions: 17, 85, 86 87, 88, and/or 89 (cellulase numbering).

19. A *Humicola insolens* cellulase variant, in which variant one or more of the following mutations have been introduced: D42W or D42Y, and/or L70Y.

20. A *Thielavia terrestris* cellulase variant, in which variant one or more of the following mutations have been introduced: P19A, G20K, Q44K, N48E, Q119H, and/or Q146 R.

21. A *Pseudomonas fluorescens* cellulase variant, in which variant one or more of the following mutations have been introduced: Y4R, H15S, N119Q, and/or Q146R.

22. A *Crinipellis scabella* cellulase variant, in which variant one or more of the following mutations hav been introduced: V4R, T132a*, Q133D, and/or Q146R.

23. *Thielavia terrestris*/Q119H.

TITLE: CELLULASE VARIANTS

ABSTRACT

The present invention relates to cellulase variants derived from a parental cellulase by substitution, insertion and/or deletion, which variant has a catalytic core domain, in which the variant at position 5 holds an alanine residue (A), a serine residue (S), or a threonine residue (T); at position 6 holds a threonine residue (T); at position 7 holds an arginine residue (R); at position 8 holds a phenylalanine residue (F), or a tyrosine residue (Y); at position 9 holds a phenylalanine residue (F), a tryptophan residue (W), or a tyrosine residue (Y); at position 10 holds an aspartic acid residue (D); at position 119 holds a holds a histidine residue (H); and at position 121 holds an aspartic acid residue (D).